

Nondestructive Analyses in Monitoring the Ripening Progress of Apple Fruit Grown in Subtropical Areas

Lucimara Rogéria Antonioli^{1,*}; Elenilson Godoy Alves Filho²; Leo Rufato³; Luciano Gebler⁴

^{1,*} Embrapa Uva e Vinho. Caixa Postal 130, 95701-008, Bento Gonçalves, RS, Brasil

² Universidade Federal do Ceará, Campus Pici, Departamento de Engenharia de Alimentos, 60440-900 Fortaleza, CE, Brasil

³ Universidade do Estado de Santa Catarina. Campus Lages, Departamento de Agronomia, 88520-000, Lages, SC, Brasil

⁴ Embrapa Uva e Vinho, 95200-000, Vacaria, RS, Brasil

*Corresponding author: lucimara.antonioli@embrapa.br

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Keywords— VIS/NIR spectroscopy, index of absorbance difference (I_{AD}), flesh firmness, soluble solids content, harvest time prediction.

Abstract— This study evaluated the correlation between the nondestructive index of absorbance difference (I_{AD}), obtained via DA-Meter, and conventional destructive quality attributes to determine the ripening of 'Fuji Suprema' apples in subtropical Brazil. During the 2018/2019 and 2019/2020 growing seasons, trials investigated several harvest parameters—including orchard row, tree face, height of fruit collection, days after full bloom (DAFB), and sunlight exposure—against attributes like skin color, flesh firmness, soluble solids, titratable acidity, starch index, and seed color. The results showed that the DA-Meter effectively monitors ripening evolution and establishes harvest onset. Ripening monitoring should begin between 142 and 147 DAFB, with readings taken strictly on the sunlit side of the fruit. Ultimately, the strong correlation between I_{AD} and both flesh firmness and soluble solids content demonstrates that this nondestructive index can successfully replace traditional destructive evaluations for apple maturation assessment.

I. INTRODUCTION

Brazilian apple production is based on Gala and Fuji varieties and their clones (Fioravanço et al., 2010). 'Fuji Suprema' was identified in Brazil from a spontaneous mutation of 'Fuji', which was obtained in Japan in 1939. 'Fuji Suprema' apple fruit have a dark red skin color, without or with a very discrete striped pattern. The skin color develops early, from 30-40 days after full bloom (DAFB). Fruit are medium to large in size, with an oblate to globose shape and cream to slightly yellowish color flesh (Fioravanço et al., 2010). Some quality attributes from 'Fuji Suprema' apple fruit on the dwarfing rootstocks M9 are: soluble solids content (SSC) of 15.87 °Brix, titratable acidity (TA) of 4.7 cmol L⁻¹, flesh firmness of 15.13 lb (67.30 N), and skin overcolor (a*) of 22.86 (Fioravanço et al., 2010). Intense red colored fruit varieties are the focus in

the modern apple breeding programs around the world, and the most valued for worldwide marketing (Dini et al., 2019). However, greater monitoring is required to determine the optimal ripening stage for the harvesting of such varieties (Fioravanço et al., 2010).

In general, immature or overripe fruits have lower marketing value and financial return, as well as waste of resources. Therefore, early evaluation of fruit ripening on the trees and the prediction of harvest time can reduce the waste and contribute to global food security (Li et al., 2018).

Nondestructive methods that allow the determination of fruit ripening on the tree have several advantages compared with the traditional destructive ones, like high-throughput assessment, simultaneous multiple measurements and real-time decision making (Li et al., 2018). The VIS/NIR spectroscopy methods can be

especially useful in indicating the ripening stage for harvest of fruits, in order to reduce losses and waste resulting from the harvest of immature or overripe fruits (Costa et al., 2017). The DA-Meter is a portable, user-friendly device that uses spectroscopy for measuring chlorophyll 'a' in the skin and flesh of the fruit by the difference between the absorbance values at 670 nm and 720 nm. The instrument gives an index of absorbance difference (I_{AD}) between the two wavelengths (Ziosi et al., 2008, Costa et al., 2017). Since large differences in fruit ripening rate pattern and specific characteristics in the pericarp can be found, the DA-Meter response is cultivar dependent (Reig et al., 2012), as well as the relationship between I_{AD} and the used harvest indexes, like flesh firmness, and soluble solids content. In addition, the climatic conditions of different production areas interfere with the catabolism of chlorophyll during fruit ripening, which implies the need to establish a model for each cultivar in each geographic region (DeLong et al., 2020).

The nondestructive DA-Meter device has been widely evaluated in apple orchards in order to monitor the ripening evolution, define the harvest time (Costamagna et al., 2013; DeLong et al., 2014; Magro et al., 2018), establish a relationship between the I_{AD} and the quality attributes at harvest and after storage (Nyasordzi et al., 2013; Toivonen & Hampson, 2014), as well as to develop optimum harvest maturity models that indicate the window for high commercial quality (DeLong et al., 2014; DeLong et al., 2016; DeLong et al., 2020).

Our study questioned a correlation between the I_{AD} , obtained in a nondestructive way from the DA-Meter equipment, and the quality attributes, obtained from destructive methods, in order to optimize the methods commonly used to evaluate the ripening of colored apple varieties. The parameters for monitoring the ripening progress were also investigated, with the aim of establishing a protocol for the use of the DA-Meter in 'Fuji Suprema' apples.

II. MATERIALS AND METHODS

A. Material

'Fuji Suprema' apples were harvested from an experimental orchard (Embrapa Uva e Vinho) located in Vacaria, Rio Grande do Sul State, Brazil (28°30'S, 50°52'W, 920 m altitude). The trees were 5 years old at the beginning of the trial. They were grafted on M9 rootstocks, and trained in a Tall Spindle system. Spacing between fruit trees was 1.25 x 4.00 m. Two trials were carried out in successive growing seasons, 2018/2019 and 2019/2020.

The full bloom was on 02/10/2018 and 07/10/2019 for those seasons, respectively.

B. Methods

Two orchard rows with 60 trees each (α and β) were selected to perform the trials. One of the rows was the edge of the orchard, in such a way the fruit were collected from its outer face, with direct sunlight, and from its inner side, facing the adjacent row. The other selected row (β), had the apples harvested only on one of the faces, regarded as the inner face. Fruit collections were performed at 3 portions of the tree, lower (up to 100 cm high), middle (between 101 and 200 cm high) and upper (from 201 cm high on).

Ten weekly fruit collections were carried out in 2018/2019 season, from 08/01/2019 to 13/03/2019 (Jan 08, 15, 22, 30, Feb 07, 13, 20, 27, and Mar 08, 13, which means 99, 106, 113, 121, 129, 135, 142, 149, 158 e 163 days after full bloom, respectively), when fruit reached complete ripening. Thirty apples were weekly collected from each of the three portions of the tree. The apples were placed inside plastic boxes (36.0 x 55.0 x 30.5 cm) which were internally protected by air bubble film in order to avoid mechanical injuries caused by impact or friction during transport of fruit.

In the lab, fruit were numbered and both sides, the more and the less colorful sides, which are the sunlit and shaded sides, respectively, were identified. Each side of the fruit was evaluated for I_{AD} , skin color, flesh firmness, soluble solids content (SSC) and titratable acidity (TA).

I_{AD} determinations were taken in an appropriate place and far from the sunlight-exposed windows since the readings from light-based instruments can be altered by stray sunlight and bright lamps (Omafra, 2014). The precise location of I_{AD} assessment in the fruit was identified in order that all the other determination, except for titratable acidity, could be made at the same location. Samples for soluble solids content measurement were taken with a pasteur pipette from the hole made by the Fruit Texture Analyzer probe. Titratable acidity was measured in the homogenized juice prepared from 10 apples, making 3 replicates of each studied condition.

The same fruit collection rows and tree faces were studied in 2019/2020 season, however only 5 fruit collections were performed, starting on 10/02/2020 and ending on 17/03/2020 (Feb 10, 17, and Mar 02, 09, 17, which means 126, 133, 147, 154, and 162 days after full bloom, respectively). The samples consisted of 20 fruit that were picked from the middle and lower portions of the tree. Apples were collected and packed in the same way as the previous year in order to protect them during transportation.

Fruit identification and assessment of I_{AD} , skin color, flesh firmness, and soluble solids content were carried out as in the previous growing season. The titratable acidity was evaluated in juice samples made of 10 apples. Starch index and seed color were further assessed for each studied condition.

Nondestructive evaluation of fruit ripening based on the level of chlorophyll in the skin was carried out by the DA-Meter, which was developed and patented by University of Bologna (DA-Meter 53500, Turoni, Italy). Destructive quality attributes were carried out as following: skin color (CIE LAB color space) determined by Tristimulus colorimeter (CR 400, Konica Minolta, Japan), flesh firmness (N) with a Fruit Texture Analyzer (GÜSS, Strand South Africa) equipped with a 11 mm cylindrical probe; SSC (°Brix) with a digital refractometer (HI96801, Hanna Instruments, USA); TA (% malic acid) by titrating 10 mL of apple juice with 90 mL of distilled water to pH 8.1 with 0.1N NaOH, using a digital burette (Digitrate Pro, VWR, USA). Starch index (color reaction score from 1 to 5 scale) by the immersion of the top half of the apple in a solution of iodine, potassium iodide and distilled water. The staining pattern was scored against a reference table of images, where 1 represents maximum starch content and 5 indicates total starch hydrolysis. Seed color (score from 1 to 4 scale) by visual evaluation where 1 defines immature seeds (cream color) and 4 indicates mature seeds (brown color).

C. Statistical analysis

Multivariate statistical analysis

One numerical matrix was created separately considering each harvest year (2019 and 2020). The apple fruit matrix from the 2019 season was composed of 180 samples, and each one was the average of approximately 25 replicates, revealing the high samples representativeness. The following fruit collection parameters were evaluated: orchard rows (α and β), trees face (inner and outer based in the next orchard row); height of fruit collection on trees (inferior, middle, and superior regions); picking days as days after full bloom (99, 106, 113, 121, 129, 135, 142, 149, 158 and 163 days) and fruit sides (sunlit and shaded). Eight fruit attributes were measured: I_{AD} index, flesh firmness, soluble solids content, and skin color related to L^* , a^* , b^* , C^* , and Hue angle.

In 2020, the apple fruit matrix was composed of 60 samples from the average of approximately 20 replicates, also revealing the sample's representativeness. Differently from the 2019 season, a total of 5 picking days were performed in 2020 for the fruit evaluation (126, 133, 147, 154, and 162 days after the full bloom), and the same fruit collection parameters for 2019 were considered. However,

ten attributes were analyzed: I_{AD} index, flesh firmness, soluble solids content, skin color related to L^* , a^* , b^* , C^* , and Hue angle, starch index (SI), and seed color.

Additionally, numerical matrices were created to analyze the titratable acidity from apple juice samples. The matrices were created separately for each harvest year, also totaling 180 samples for 2019 and 60 samples for 2020. The I_{AD} and titratable acidity were the measured attributes for those numerical matrices construction.

Each numerical matrix was separately imported by the PLS-Toolbox™ program (version 8.6.2, Eigenvector Research Incorporated, Manson, WA USA) for unsupervised chemometric analysis by Principal Component Analysis (PCA) in order to reveal the main samples variability based on the fruit collection parameters. For chemometric evaluation, the data was autoscaled (mean-centered with subsequent variance scaling), and the Singular Value Decomposition (SVD) algorithm was applied to decompose the complex matrix in scores and loadings under confidence level of 95%.

Multivariate regression analysis

In order to predict apple attributes achieved by destructive methods (as flesh firmness and soluble solids content) using data from a nondestructive technique (as I_{AD}), the correlation of the apple fruit attributes and fruit collection parameters was developed by multivariate regression modeling by Partial Least Squares (PLS) using the same PLS-Toolbox™ software. Then, the flesh firmness and soluble solids content data were used as X matrix, which was correlated with I_{AD} as Y matrix. The SIMPLS (Simplified PLS) algorithm was applied for modeling using the Venetian Blinds method for cross-validation. The number of Latent Variables (LV) and models' quality was selected according to low values of errors achieved by the Root Mean Square Error of Calibration (RMSEC) and Cross-Validation (RMSECV), low cross-validated bias values (average difference between the estimator and real values during the cross calibration), and high determination coefficients (R^2) achieved in calibration (R^2_{cal}) and cross-validation (R^2_{CV}).

Analysis of Variance

In order to statistically certify the differences between the fruit and juice samples characteristics, the combined uncertainty was estimated based on approximately 25 fruit replicates from 2019, and 20 replicates from 2020 season. The results were evaluated using the analysis of variance (ANOVA) single factor under significance level of 0.05, Tukey's test for means comparison, and Levene to test the variance homogeneity among the samples' groups.

III. RESULTS AND DISCUSSION

A. Data variability according to the fruit collection parameters

Based on the source of the fruit attributes and the experimental aim, the I_{AD} , flesh firmness, and the soluble solids content were evaluated separately from the skin color parameters (L^* , a^* , b^* , C^* , and Hue angle). Therefore, one PCA evaluation was developed for each type of data: Figures 1a and 2a illustrate the scores and loadings biplot of

the I_{AD} , flesh firmness, and SSC as attributes for 2018/19 and 2019/20 growing seasons, respectively; Figures 1b and 2b show the scores and loadings biplot from the color parameters (L^* , a^* , b^* , C^* , and Hue angle) based on the fruit side to the sunlight (sunlit and shaded), respectively; and Figures 1c and 2c present the scores and loadings biplot also from the color parameters based on the picking days, respectively. The other fruit collection parameters (orchard row, tree face, and tree height for fruit collection) were not relevant for the samples' discrimination.

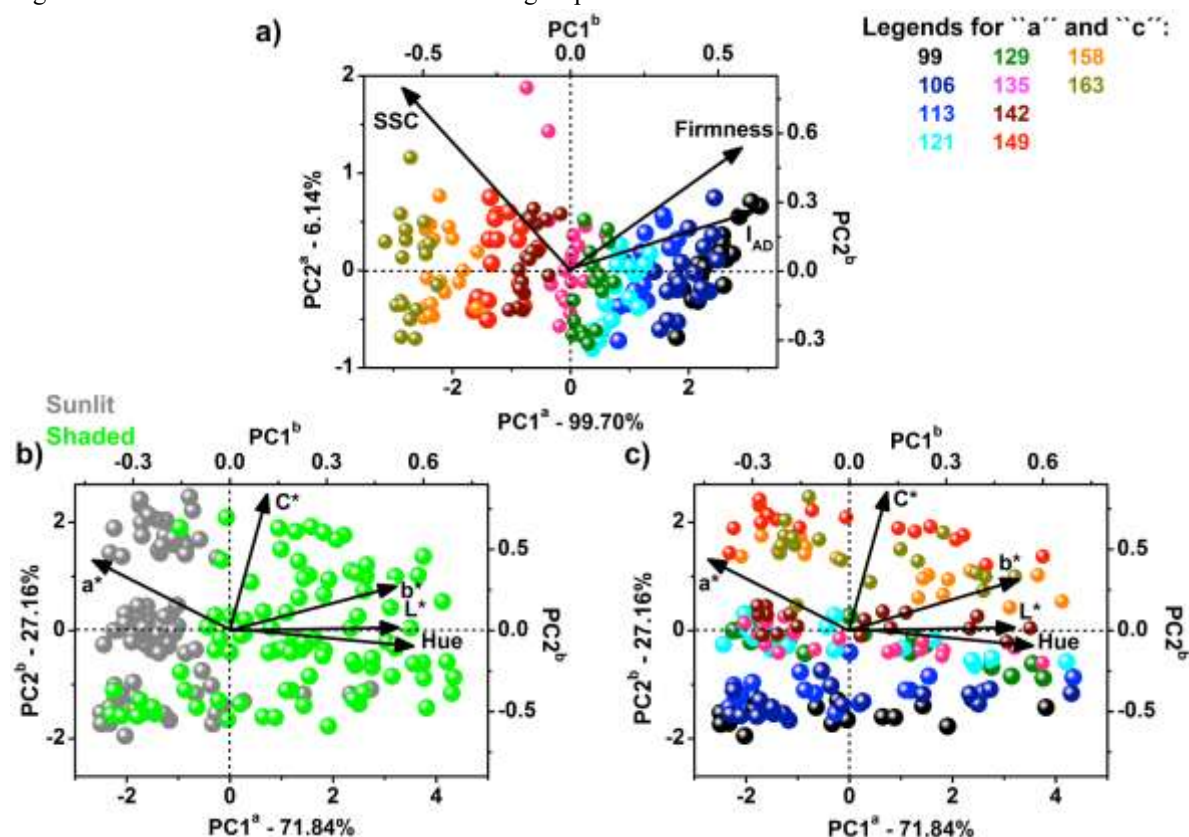


Fig.1. PCA biplots (scores and loadings) of the I_{AD} , flesh firmness, and SSC from apple fruit harvested in 2019 season considering the DAFB (99, 106, 113, 121, 129, 135, 142, 149, 158 and 163) (a) and skin color parameters (L^* , a^* , b^* , C^* , and Hue angle) based on the fruit side to the sunlight (sunlit and shaded) (b), and on the DAFB (c).

Regardless of the harvest year (2019 or 2020), the picking days relating to DAFB was the main factor for the fruit discrimination according to the I_{AD} , flesh firmness, and soluble solids content. This samples variability was retained on the PC1 axis: apples gathered in the beginning of the studied period (99, 106, 113, 121 e 129 DAFB for 2019 harvest, and 126, 133 DAFB for 2020 harvest) clustered in positive scores by high values of flesh firmness and I_{AD} ; and apples picked up in the ending (142, 149, 158, and 163 DAFB for 2019 harvest, and 154, 162 DAFB for 2020 harvest) in negative scores by high values of soluble solids content. The intermediary fruit collection in the 2019 harvest (135 DAFB) clustered in null scores by

intermediary values of flesh firmness, I_{AD} , and SSC. The same was observed on apples gathered 147 DAFB in the 2020 harvest, although those values showed some tendency to cluster in negative scores of PC1.

The skin color parameters were also relevant attributes for apple fruit discrimination regardless of the harvest year (2019 or 2020). Based on the fruit side to the sunlight (Fig. 1b for 2019, and Fig. 2b for 2020 season), PC1 was the main factorial axis for the fruit discrimination. Apple fruit samples clustered at positive scores by high values of b , L , and Hue angle; and samples clustered at negative scores by high values of a . The C , seed color (SC)

and starch index (SI) were weakly correlated for samples discrimination according to the fruit side to the sunlight. However, the PC2 axis revealed an important correlation of C (Fig. 1c for 2019, and Fig. 2c for 2020 season), SC and SI (Fig. 2c for 2020) with the picking days relating to

DAFB, with apple samples harvested at the end of the studied period clustered in positive scores by high values of C, SC and SI (those two last attributes only for the 2020 harvest), together with high values of a and b. The L and Hue angle were weakly correlated with the DAFB.

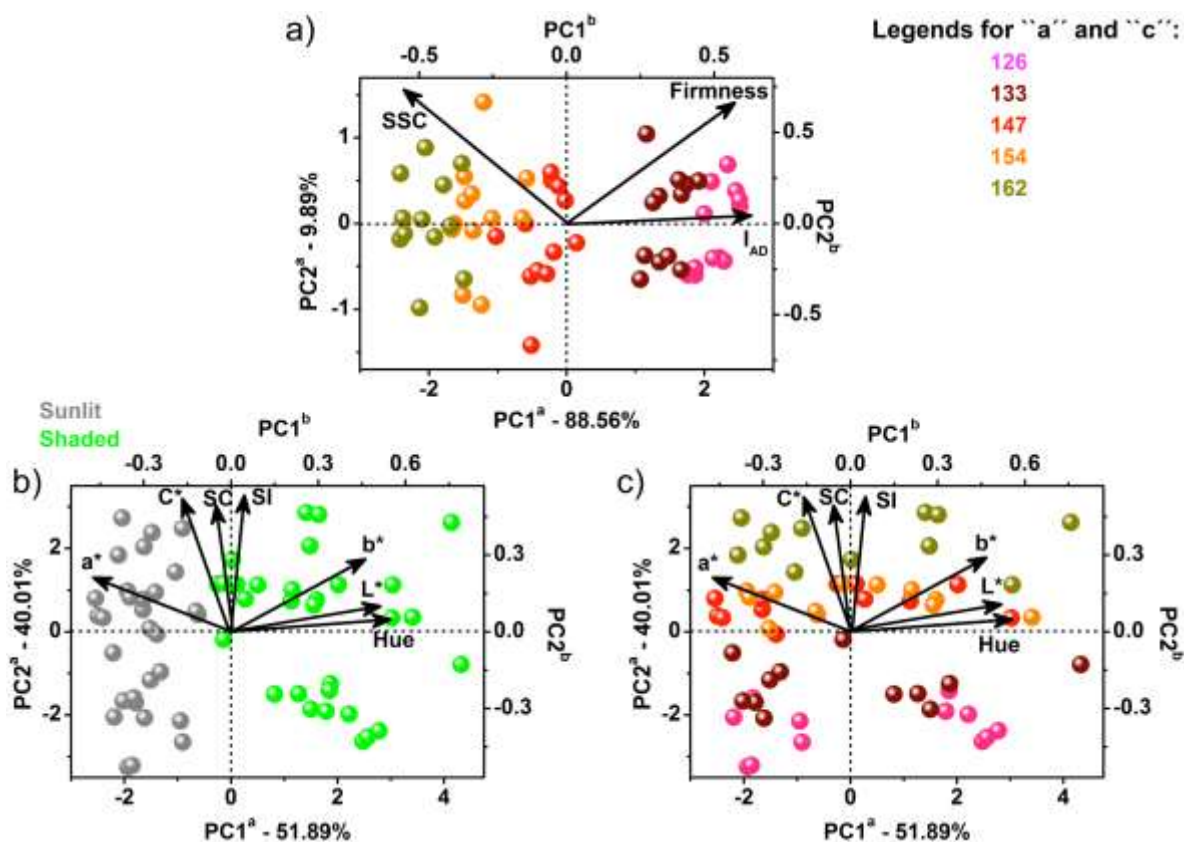


Fig.2. PCA biplots (scores and loadings) of the I_{AD} , flesh firmness, and SSC from apple fruit harvested in 2020 season considering the DAFB (126, 133, 147, 154, and 162) (a), skin color parameters (L^* , a^* , b^* , C^* , and Hue angle), seeds color (SC), and starch index (SI) based on the fruit side to the sunlight (sunlit and shaded) (b), and on the DAFB (c).

Analysis of Variance (ANOVA single factor) was developed for each sample attributes in order to certify the variability detected by PCA: I_{AD} , flesh firmness, soluble solids content, and skin color related to L^* , a^* , b^* , C^* , and Hue angle for 2019 and 2020 harvest, with addition of starch index and seed color for 2020. The results are illustrated as box plot in Figures 3 and 4, and 5 and 6 for the harvest years 2019 and 2020, respectively. For both harvest years, the ANOVA results statistically certify the decrease of the I_{AD} (Fig. 3a and 5a) and flesh firmness (Fig. 3b and 5b), and the increase of soluble solids content (Fig. 3c and 5c) as the days pass after the full bloom, as well as the differences of the skin color parameters (L^* , a^* , b^* , C^* , and

Hue angle) in the sunlit and shaded apple sides (Fig. 4 and 6). In addition, increases in the attributes seed color and starch index were also corroborated by ANOVA in apple fruit harvested in the 2020 season (Fig. 5e and 5f).

As detected in apple fruit samples, ANOVA also certified the I_{AD} decrease with the passing of time in the apple juice samples (data not shown), regardless of the harvest year (2019 and 2020), orchard row, trees face based in the next orchard row, height of fruit collection on trees, and fruit side to the sunlight. However, the titratable acidity did not show significant correlation with the investigated fruit collection parameters.

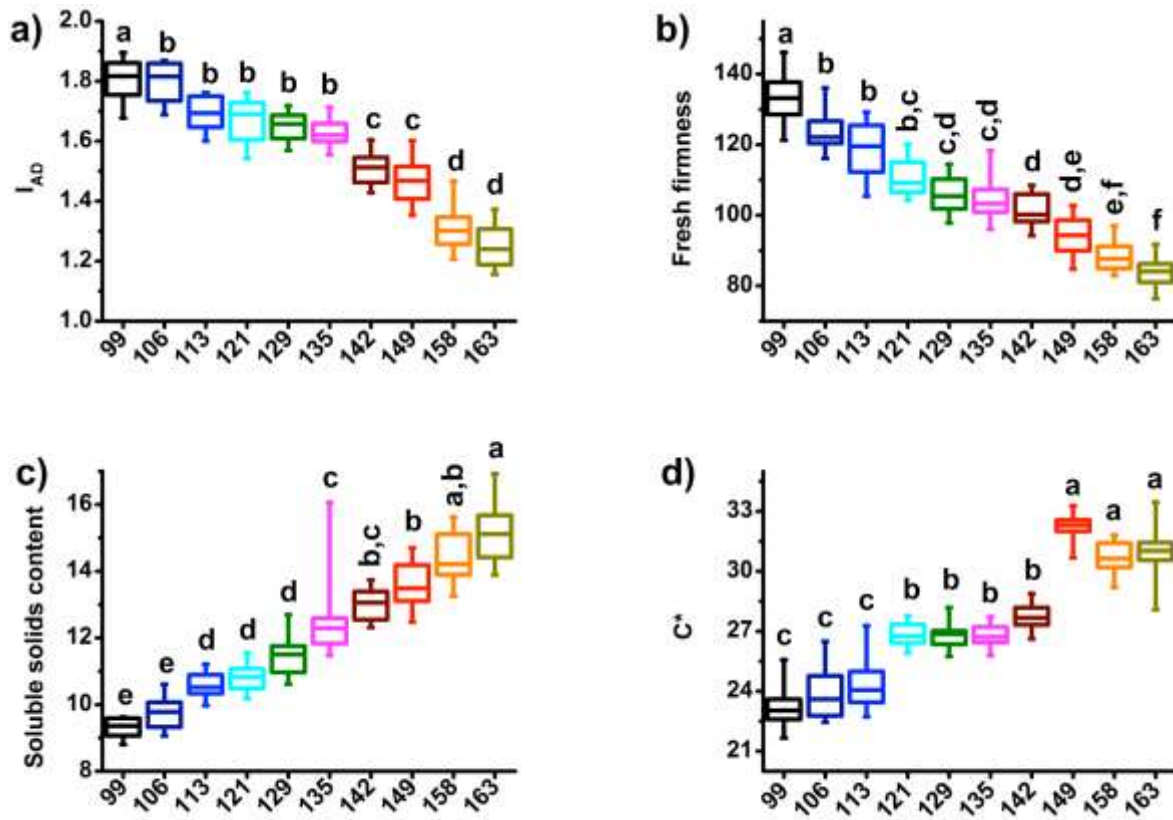


Fig.3. Box plot of the following parameters from apple fruit harvested in 2019 season: a) I_{AD} ; b) flesh firmness; c) soluble solids content; d) C^* .

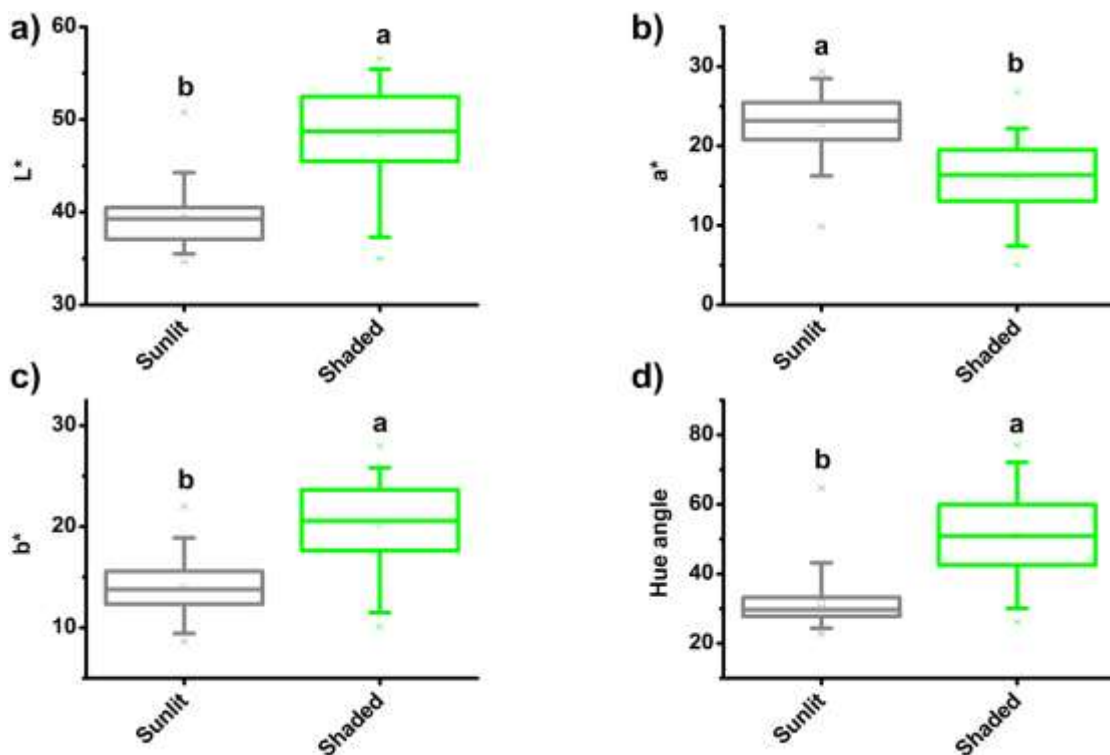


Fig.4. Box plot of the skin color parameters from apple fruit harvested in 2019 season: a) L^* ; b) a^* ; c) b^* ; and d) Hue angle.

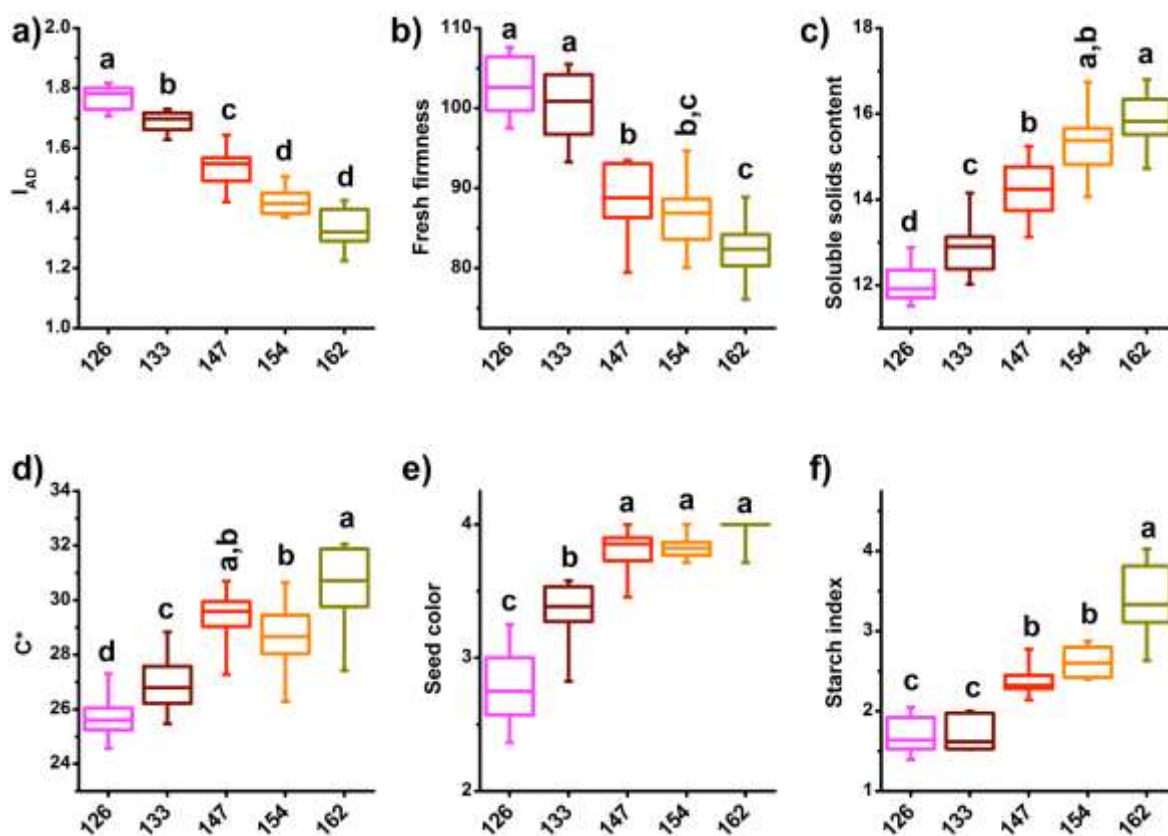


Fig.5. Box plot of the following parameters from apple fruit harvested in 2020 season: a) I_{AD} ; b) flesh firmness; c) soluble solids content; d) C^* ; e) seed color; f) starch index.

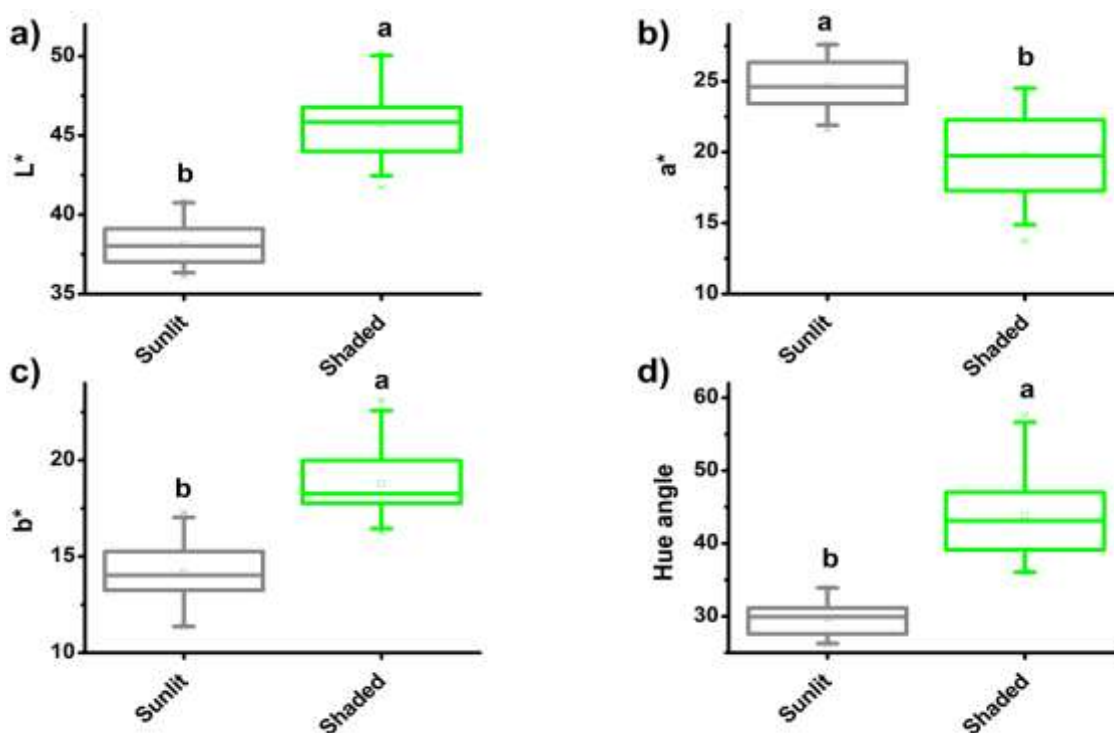


Fig.6. Box plot of the skin color parameters from apple fruit harvested in 2020 season: a) L^* ; b) a^* ; c) b^* ; and d) Hue angle.

B. Multivariate classification analysis

In order to evaluate the apples variability achieved by clustering in PCA corroborating the ANOVA results, four multivariate classification modeling by PLS-DA and Confusion Matrix analysis were developed considering picking days as DAFB and fruit sides as categorical class: 99, 106, 113, 121, 129, 135, 142, 149, 158, and 163 DAFB for 2019 harvest; sunlit and shaded for 2019 harvest; 126, 133, 147, 154, and 162 DAFB for 2020 harvest; sunlit and shaded for 2020 harvest. For fruit classification based on picking day, the I_{AD} , flesh firmness, soluble solids content, and C^* were used as quality

attributes, with addition of the seed color and starch index for 2020 harvest. For fruit classification based on the exposed fruit side to the sunlight, the color parameters L^* , a^* , b^* , and Hue angle were selected as quality attributes. Tables 1 and 2 describe the statistical performance of the Confusion Matrix based on the cross-validated (CV) prediction capacity for picking day and fruit side to the sunlight, respectively. **Table 1.** Confusion Matrix results of apples based on the picking day as DAFB according to the I_{AD} , flesh firmness, soluble solids content, and C^* for 2019 and 2020, with addition of seed color and starch index to 2020 harvest year.

Table 2. Confusion Matrix results of apples based on the fruit side to the sunlight according to the color parameters L^* , a^* , b^* , and Hue angle.

		REAL CLASS									
PREDICTED CLASS	Days after full bloom (2019)	99	106	113	121	129	135	142	149	158	163
	99	11	17	2	0	0	0	0	0	0	0
	106	7	0	12	0	0	0	0	0	0	0
	113	0	1	0	15	0	1	0	0	0	0
	121	0	0	4	0	14	11	1	0	0	0
	129	0	0	0	0	0	1	12	0	0	0
	135	0	0	0	0	0	0	0	0	0	0
	142	0	0	0	3	4	5	0	0	0	0
	149	0	0	0	0	0	0	5	0	1	0
	158	0	0	0	0	0	0	0	17	0	8
	163	0	0	0	0	0	0	0	1	17	10
	Total	18	18	18	18	18	18	18	18	18	18

		REAL CLASS				
PREDICTED CLASS	Days after full bloom (2020)	126	133	147	154	162
	126	12	10	0	0	0
	133	0	0	0	0	0
	147	0	2	0	0	0
	154	0	0	12	0	0
	162	0	0	0	12	12
	Total	12	12	12	12	12

		REAL CLASS	
PREDICTED CLASS	Fruit side (2019)	Sunlit	Shaded
	Sunlit	82	19
	Shaded	8	71
	Total	90	90

		REAL CLASS	
PREDICTED CLASS	Fruit side (2020)	Sunlit	Shaded
	Sunlit	30	0
	Shaded	0	30
	Total	30	30

In general, the models showed high classification performance to discriminate apples according to the I_{AD} , flesh firmness, soluble solids content, and C^* from the first pickings (from 99 to 142 DAFB) to the three last pickings (149, 158, and 163 DAFB) for 2019 harvest, and from the two first pickings (126 and 133 DAFB) to the last pickings (147, 154, and 162 DAFB) for 2020 harvest with addition of seed color and starch index (Table 1). In addition, the classification models based on the color parameters (L^* , a^* , b^* , and Hue angle) had high classification performance of the apples according to the exposure side to the sunlight (Table 2) for 2019 and 2020 harvest.

Table 3 describes the statistical parameters from the four classification methods (Tables 1 and 2), showing

the acceptable modeling performance by high sensitivity [$TP / (TP + FN)$] and specificity [$TN / (TN + FP)$] achieved on cross-validation (CV), as well as low modeling errors achieved by the RMSEC and RMSECV methods and CV bias (non-biased models): True Positive (TP); True Negative (TN); False Positive (FP); and False Negative (FN) (Sokolova & Lapalme, 2009; Lever et al., 2016).

The elevated proximity between RMSEC and RMSECV values (similarity index by $RMSEC / RMSECV > 0.75$) corroborated the modeling quality for apples classification (De Oliveira et al., 2014; Freitas et al., 2018). Moreover, the outliers were determined by visual inspection of Q residuals $\times$ Hotelling's T^2 plot, and despite some samples presented high Q residuals and/or influence on modeling (Hotelling's T^2), they did not affect the robustness of the apples' classification.

C. Regression analyses

A model to predict data from destructive techniques using data from a nondestructive technique as I_{AD} was established. Therefore, due to the reproducibility of the I_{AD} , flesh firmness, soluble solids content, and C^* , multivariate (by PLS) and univariate regression analyses were developed considering the total dataset (2019 and 2020 harvest year). Figure 7 shows the regression results of the apples harvested in 2019 and 2020 seasons together: measured and predicted flesh firmness, soluble solids content, and C^* based on the I_{AD} measurement achieved by PLS method, and regression analysis of the I_{AD} and flesh firmness, soluble solids content, and C^* .

Table 3. Statistical parameters of the multivariate classification models.

Parameters	Picking days after full bloom (2019)	Picking days after full bloom (2020)	Exposure fruit side to the sunlight (2019)	Exposure fruit side to the sunlight (2020)
LV number	1	1	1	1
Captured variance (%)	89.47	89.09	82.75	88.49
RMSEC ^a	0.26	0.34	0.38	0.23
RMSECV ^b	0.30	0.37	0.46	0.24
RMSEC/RMSECV ^c	0.87	0.92	0.83	0.96
CV Bias ^d	-0.012	-0.007	0.015	-0.007
Sensitivity	0.83	0.92	0.92	1.00
Specificity	0.87	0.81	0.60	1.00

^a Root Mean Square Error of Calibration; ^b Root Mean Square Error of Cross Validation; ^c Similarity index; ^d Average difference between the estimator and real values in during the cross calibration.

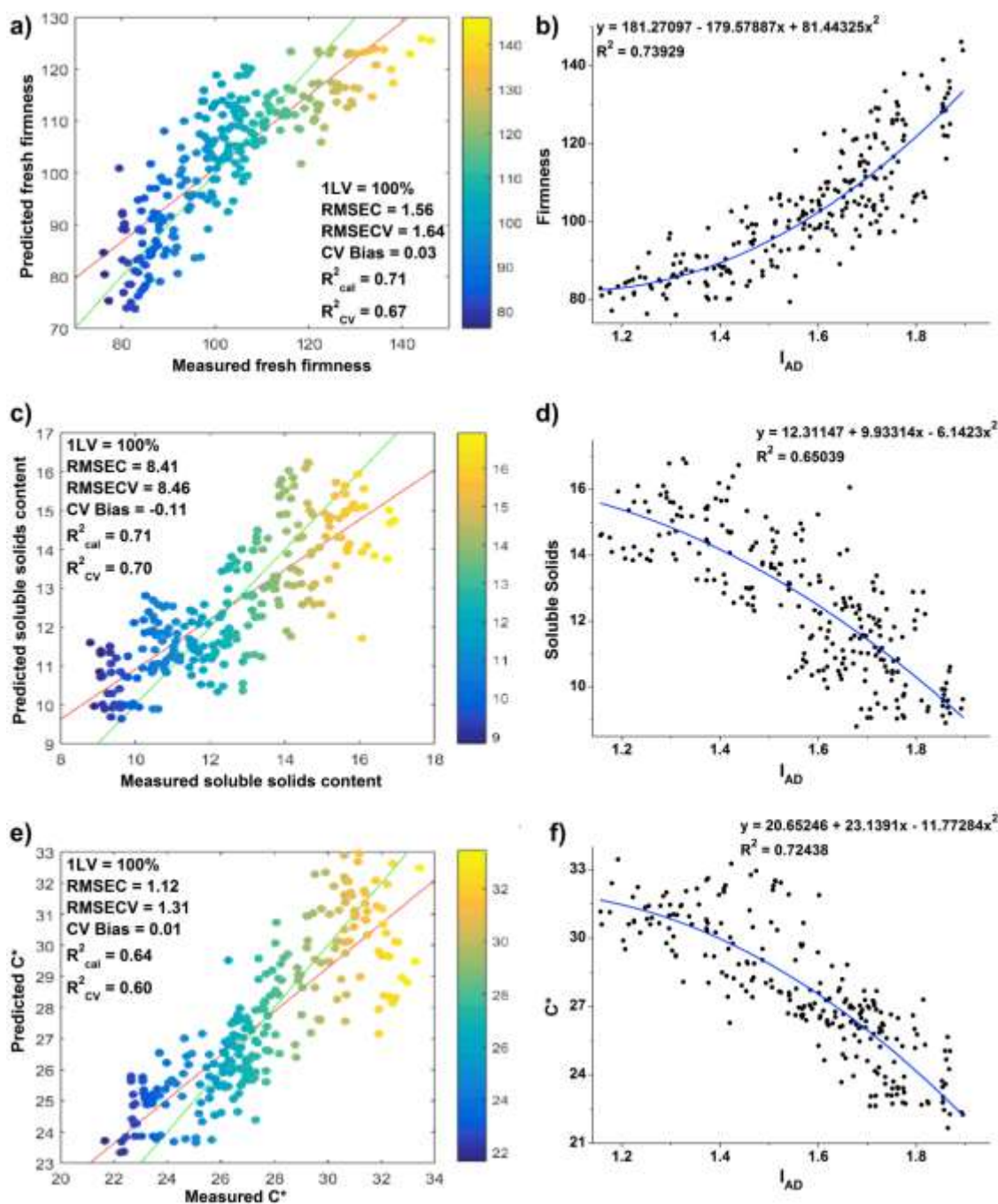


Fig.7. Regression results of the apples harvested in 2019 and 2020 season: a) real (red) and ideal (green) regression curves of the measured and predicted flesh firmness based on I_{AD} by PLS; b) quadratic regression analysis of the I_{AD} and flesh firmness; c) real (red) and ideal (green) regression curves of the measured and predicted soluble solids content based on I_{AD} by PLS; d) quadratic regression analysis of the I_{AD} and soluble solids content; e) real (red) and ideal (green) regression curves of the measured and predicted C^* based on I_{AD} by PLS; f) quadratic regression analysis of the I_{AD} and C^* .

The accuracy of the multivariate regression models was evaluated based on low errors achieved by the RMSEC and RMSECV methods with the respective high similarity index (proximity between the RMSEC and RMSECV values), low bias values in cross-validated models, and high coefficients of determination on calibration (R^2_{cal}) and cross-validation (R^2_{cv}) with the

proximity between them, since R^2 indicates the percentage of the variance in the Y matrix (independent variables) that is accounted by the X matrix (dependent variables): R^2 between 0.50 and 0.65 indicates that more than 50% of the variance in Y matrix is accounted by the variance in X (acceptable discrimination between high and low concentrations); R^2 between 0.66 and 0.81 indicates

approximate quantitative predictions; R^2 between 0.82 and 0.90 indicates good prediction; and R^2 above 0.91 indicates an excellent model (Williams et al., 2019; Saeys et al., 2005).

Therefore, all the multivariate regression models by PLS are approximate to quantitative predictions using only 1 LV reaching 100% of the total variance, with well-adjusted methods by high proximity between the RMSEC and RMSECV values (RMSEC / RMSECV above 0.75) (De Oliveira et al., 2014; Freitas et al., 2018). The regression quality of the multivariate models was corroborated by the coefficients of determination (R^2) with significant regression coefficients ($P < 0.05$) reached by the quadratic regression between the I_{AD} and flesh firmness (Fig. 7b) of 0.74, I_{AD} with soluble solids content (Fig. 7d) of 0.65, and I_{AD} with C^* (Fig. 7f) of 0.72 (Nyasordzi et al., 2013; Zhang et al., 2017).

IV. DISCUSSION

The studied set of fruit collection parameters revealed that only the picking day (DAFB), and the fruit side influenced the quality attributes of 'Fuji Suprema' apples, which confirm them as important parameters to be considered in the studies of ripening monitoring to establish the beginning of the harvest season. The height of fruit collection and the tree face were not significant in the samples' discrimination, which reduces the requirement for data collection targeting and simplifies the fruit sampling.

The number of days after the full bloom is a parameter usually monitored in the apple production, but the combination of several ripening indicators gives more reliability for establishing the beginning of fruit harvest. Chemical and physical quality parameters such as flesh firmness, starch index, soluble solids content, titratable acidity, seed color, and internal ethylene concentration are good indicators of ripening evolution but all of them are obtained from destructive methods which demand time and financial resources. The background skin color is another good ripening indicator for bicolored apples, however it does not work for apples that have the surface almost recovered by a solid red color, like 'Fuji Suprema' apples. The use of proximal monitoring systems, such as the DA-Meter equipment, can reduce costs and provide agility and assertiveness to the production system.

In the growing seasons 2018/2019 and 2019/2020 the beginning of fruit collection was on January 08, at 99 DAFB and February 10, at 126 DAFB, respectively. Fruit harvested by 129 and 133 DAFB in the 2018/2019 and 2019/2020 crops, respectively, clustered in the positive portion of PC1. The further away from full bloom, the

greater the distribution of samples towards the more negative PC1 values.

'Fuji Suprema' bloom usually takes place from the 2nd half of September to the 1st half of October in Rio Grande do Sul climate conditions, the ripening happens between mid-March and end of April, and the fruit development occurs between the two phenological stages (Fioravanzo et al., 2010). In the studied growing seasons, the full bloom was noticed in the 1st week of October and the commercial ripening stages were reached on 13/03/2019 at 163 DAFB (2018/2019), and 17/03/2020, at 162 DAFB (2019/2020), dates of the last fruit collections. It shows that the phenological stages were as expected for the variety and producing area.

Considering the fruit collection distribution where the apples clustered in positive scores of PC1 are represented by high flesh firmness and high I_{AD} and those clustered in negative scores are represented by high SSC, it can be inferred that the period between 142 and 147 DAFB is the best one to start monitoring the ripening progress, since the changes related to maturation and ripening are intensified. From that period onwards, some attributes show drastic changes after a period of stability (such as skin chroma), while other attributes undergo gradual changes, like SSC.

In the given period of 142-147 DAFB for starting the ripening monitoring, it is expected that the I_{AD} of about 1.50 will reach 1.25 at harvest, the flesh firmness will decrease from 95 N to 83 N, the SSC of 13 °Brix will reach 15.8 °Brix, and the skin chroma will go from 28 to 31. It is still expected that the seeds will be completely brown and the fruit will reach a starch index higher than 3 at the commercial harvest (162 DAFB). The uniformity in the maturation progress mainly in the last 3 fruit collections, i.e., when the apples are reaching commercial maturity, is an indication that the harvest can be done all at once, avoiding re-harvesting in the same area.

The fruit side was also important in the discrimination of the samples in the PC1 axis. The less colorful shaded side was posed mainly by b^* , L^* and Hue angle of skin color, whereas the sunlit side was better represented by the parameter a^* of the skin color, whose axis varies from green to red. Lower lightness, and redder hues (lower Hue angles) on the sunlit side had been previously reported for apples (González-Talice et al., 2013). The distinction between both sides of the apples during several fruit collections over two harvest years was feasible, and the models had high performance of classification, despite the red color that develops very early on 'Fuji Suprema' apples and covers almost all the fruit surface. The same discrimination of the samples in the PC2

axis reveal that the chroma (C) from the skin color, as well as the seed color and the starch index are important attributes as maturation progresses, especially for fruit harvested from 147-149 DAFB.

While the chroma of the skin proved to be relevant over time, this attribute was weakly correlated to the fruit side in our study. There is no common understanding in the literature about which fruit side, shaded or sunlit, should be measured on apples. Most of them use a mean value that is obtained from sampling the fruit at two equidistant points in the equatorial region, which may not properly represent either side sampled. On the other hand, Magro et al. (2018) evaluated the I_{AD} and the quality attributes in the transition portion between the red skin color and the background green color of 'Maxi Gala' apples. Another study suggests that the deeply shaded side must be avoided because it contains less chlorophyll than the light-exposed side of the apple. In the same way, the exposed side to the sunlight must be avoided as well, since this side will likely suffer lethal or sub-lethal sunscald or heat injury, which causes chlorophyll bleaching and consequently much lower chlorophyll values than on the sides not exposed to direct sunlight (Omafra, 2014). The authors recommend the north-facing side of the fruit for measuring the apple, since it is not exposed to direct sun or deep shade (Omafra, 2014).

Higher chroma was observed on the sunlit side of apples and it was positively related with chlorophyll a (González-Talice et al., 2013). Since the index of absorption (I_{AD}) is based on the difference between the absorption at two wavelengths around the chlorophyll a peak (670 nm and 720 nm), a polynomial model between I_{AD} and chroma could be established ($C^* = 20.65 + 23.14 I_{AD} - 11.77 I_{AD}^2$; $R^2 = 0.72$) in our study. We suggest that the I_{AD} measurement be standardized preferably on the apple sunlit side, since the proximal monitoring technology may, in a near future, be embedded in more robust systems, in order to collect the data when passing through the crop rows.

The most important correlations found in our study that allow the substitution of ripening indicators obtained from destructive methods by the I_{AD} , a nondestructive method of data collection were between I_{AD} and flesh firmness (Flesh firmness (N) = $181.27 - 179.58 I_{AD} + 81.44 I_{AD}^2$; $R^2 = 0.74$) and between I_{AD} and soluble solids content (SSC (°Brix) = $12.31 + 9.93 I_{AD} - 6.14 I_{AD}^2$; $R^2 = 0.65$). Good correlations between the I_{AD} values and other harvest indices obtained from destructive analysis (soluble solids content, flesh firmness, starch-iodine index, titratable acidity, and internal ethylene concentration) were found on 'Maxi Gala' apples (Magro et al., 2018), 'Starking', 'Granny Smith' and 'Pink Lady' (Nyasordzi et al., 2013), 'Gala' (Costamagna et al., 2013), 'Braeburn' and 'Cripps

Pink' (Zanella et al., 2013) and 'Honeycrisp', 'Ambrosia', and 'Minneiska' (DeLong et al., 2014; Delong et al., 2016; Delong et al., 2020). Monitoring the harvest indices are crucial in the harvest planning and in predicting the long-term storage potential of apples. Since preharvest applications of the plant growth regulators that inhibit ethylene production like ReTain (aminoethoxyvinylglycine, AVG) and Harvista (1-methylcyclopropene, 1-MCP) affects the correlations between I_{AD} and the harvest indices (Doerflinger et al., 2016; Blanke & Kunz, 2018), our findings are useful for the harvest prediction of 'Fuji Suprema' apples with no pre-harvest treatment with plant growth regulators.

V. CONCLUSION

The DA-Meter can be used to monitor the ripening evolution of 'Fuji Suprema' apples grown in Rio Grande do Sul State, Brazil, in order to establish the beginning of the harvest. Monitoring the evolution of ripening with the DA-Meter should start from 142 to 147 DAFB. The readings must be obtained on the sunlit side of the fruit. Finally, the correlation between I_{AD} and flesh firmness, and between I_{AD} and soluble solids content allows the replacement of these destructive evaluations that are commonly used to evaluate the maturation of apples by I_{AD} .

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AUTHOR CONTRIBUTIONS

LRA: Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition EGAF: Formal analysis, Writing – original draft. LR: Resources LG: Conceptualization, Methodology, Resources, Funding acquisition.

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