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Spectroscopic Analysis of Proximal Leaves as a Method for Studying Nectarine Ripening

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Cite This: https://doi.org/10.1021/acsagscitech.4c00760

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ABSTRACT: Traditional methods for fruit quality assessment are labor-intensive, destructive, and result in the loss of marketable produce. Spectroscopy, especially near-infrared (NIR) and mid-infrared (MIR), has helped in the analysis of fruit quality, despite being nondestructive, as it can leave some marks on the fruit. This study investigates the potential of NIR and MIR spectroscopy for monitoring nectarine ripening through the analysis of proximal leaves, leveraging their biochemical and physiological changes during ripening as a practical and truly noninvasive alternative to predict key fruit attributes. Spectral data were analyzed using ANOVA-Simultaneous Component Analysis (ASCA) to determine the key factors influencing spectral variability. The results indicated that the evolution of the spectra was the primary contributor to spectral changes, reflecting physiological dynamics during fruit ripening. Partial Least Squares (PLS) regression models were employed to predict key fruit properties (weight, firmness, sugar content, pH and acidity). The models showed acceptable performance for indirect prediction with R^2_{CV} values ranging from 0.4 to 0.7, RPD values from 1.41 to 1.88, and RER values from 5.56 to 10.21. Predictions were good for nectarine properties like weight and firmness, with leaf spectra effectively predicting these fruit characteristics, though predictions for acidity and pH were less robust. Key findings suggest that combining spectral data from both sides of the leaf provides models with good performance, offering a practical noninvasive alternative to destructive fruit quality analysis methods and providing valuable insights for precision agriculture. This approach has great potential to redefine ripening assessments in fruit production and monitoring practices.

KEYWORDS: *Fruit quality prediction, data fusion, Design of Experiments (DoE), precision agriculture, peach (Prunus), ripening dynamics*

1. INTRODUCTION

Nectarines (*Prunus persica* var. *nucipersica*) are among the most commercially valuable stone fruits, with a peach and nectarine global production of 25.4 million tonnes. Major producers include China, the European Union, Türkiye, and the United States, which collectively account for more than 91% of worldwide supply.[1] In Spain alone, nectarines and peaches represented approximately €306 million in export value in 2024.[2] Beyond their economic impact, nectarines are valued for their high nutritional quality and consumer preference for fresh, sweet, and firm fruit. Thus, the achievement of fruit quality is the primary objective in the horticultural industry, as it directly influences marketability, consumer satisfaction, and economic returns. Nectarines, like other stone fruits, have specific quality attributes that are highly dependent on their nutrient status throughout the growing season. High-quality fruits are characterized by desirable attributes such as firmness, sweetness, acidity, and storage potential.[3,4] Ensuring these quality traits requires precise, ongoing fruit management throughout their growth and maturation, based on physicochemical analyses that traditionally involve destructive sampling methods. These methods are labor-intensive and imply the loss of marketable fruits used for the analyses. Thus, there is an increasing demand for green, rapid, nondestructive methods to monitor and ensure fruit quality without compromising the integrity of the product.[5,6] The potential of infrared spectroscopy to serve as a nondestructive diagnostic tool is especially valuable in fruit production.

Spectroscopy has already been successfully applied to measure the ripening status of fruit, particularly nectarines. However, while considered nondestructive technique, it can leave marks on the skin of the fruit, potentially reducing the overall quality of the product. Furthermore, if the instrument is not portable, this requires harvesting some fruits during the ripening process, resulting in a loss of production. An alternative approach is to analyze the leaves proximal to the fruit,[7] which can provide valuable insights into the ripening process without affecting the fruit itself. This approach is supported by the well-documented correlation between leaf nutrient content and fruit quality, as leaves play a central role in photosynthesis and nutrient distribution processes, both of which directly impact fruit development.[8−11]

Near-Infrared (NIR) and Mid-Infrared (MIR) spectroscopy provide detailed information about the biochemical composition of leaves: MIR spectroscopy provides specific information

Received: November 26, 2024
Revised: October 11, 2025
Accepted: October 13, 2025

about molecular structures through fundamental molecular vibrational modes, while NIR spectroscopy is particularly sensitive to their overtones and combinations of fundamental vibrations.[12,13] In other words, these techniques generate a unique chemical fingerprint of the leaves,[7] enabling the detection of subtle changes in leaf metabolites that correlate with the nutritional and ripening status of the proximal fruit.

The objective of this study is to evaluate the feasibility of using the spectral characteristics of leaves located proximal to nectarines as an indirect, noninvasive tool for monitoring fruit ripening. By systematically analyzing over time the spectral data of leaves proximal to the fruit, we aim to develop a nondestructive, rapid, and reliable method for assessing fruit quality. Specifically, we aim to (i) identify the most influential factors associated with leaf position that affect their spectral features; (ii) determine whether physiological changes in leaves, using NIR and MIR spectroscopy, correlate with fruit ripening stages and (iii) compare the predictive performance of NIR and MIR spectroscopy in modeling key fruit quality parameters. This approach has the potential to transform current practices in fruit quality monitoring, providing growers with a practical tool for real-time decision-making.

2. MATERIAL AND METHODS

2.1. Samples. Nectarine leaves (*Prunus persica* var. *nucipersica*) of the 'Luciana' variety were selected for this study due to their commercial availability and regional relevance.[12] The leaves were collected from a commercial orchard located in Artesa de Lleida (41°32′20.2″N 0°42′43.7″E; Catalonia, Spain), located at an altitude of 205.15 m and approximately 60 km from the Mediterranean Sea. Twelve trees were randomly chosen and marked for the study. These trees received standard orchard management practices, including irrigation, pruning and thinning.

To get a reference for predicting nectarine characteristics through leaf analysis, nectarines were sampled at various stages throughout the ripening period (following the experimental design outlined in section 2.2) from the 12 marked trees. At the same moment, the leaves sampled were the closest to the collected nectarines and were coded and stored in plastic zip-lock bags to facilitate correlation with the corresponding nectarine data.

2.2. Experimental Design. The experimental design for the collection of leaves (and their reference nectarines) was the same as described in Ezenarro et al.[14] The sampling was designed to account for the intrinsic variability of the maturation process of nectarines due to their position: the considered factors were the vertical position ('Height') of the nectarine within the tree (either medium or high, as the low-lying nectarines were lost to frost during the flowering stage), the tree's directional orientation relative to the sun (northwest, receiving less solar radiation, or southeast, receiving more), and the proximity of the nectarine to the tree trunk ('Prominence'). Four biological replicates, i.e., four nectarine and adjacent or proximal nectarine leaves fitting these criteria, were randomly selected from the trees under study. The temporal aspect, crucial for the growth and development of nectarines, was carefully monitored by conducting weekly sampling over a period of 11 weeks beginning in the third week of May 2022, after the hardening of the nectarine stones.

Additional factors considered in the spectroscopic measurement of nectarine leaves were the two sides, the upper part (adaxial, the part directed to the sun) and the lower part (abaxial, the part underneath). As nectarine leaves were removed from the tree, each week 32 leaves that met the experimental design were collected.

2.3. Physicochemical Analysis. The physicochemical analyses used to monitor nectarine ripening are the same as described in Ezenarro et al.[14] In brief, each fruit was individually weighed, and firmness (in kg) was measured on two sides of the nectarine using a penetrometer (Fruit Pressure Tester, mod. FT-327, Italy) with an 8 mm probe. After firmness testing, each nectarine was individually blended and the juice was used to measure pH with a pH-meter (7+ series, XS Instruments, Italy), to determine total acidity (expressed as g malic acid · L^{-1}) by titration with NaOH (0.1 M) until a pH of 8.2 and to evaluate the Total Soluble Solids (TSS in °Brix) concentration using a digital refractometer (HI 96801, Hanna Instruments, United States). Due to the small volume of juice obtained in the early stages of nectarine ripening, pH measurements began in the third week (after stone hardening) and acidity measurements in the seventh week. Firmness values exceeded the equipment's 13 kg limit, making measurements impossible until the fifth week.

2.4. Near-Infrared Spectroscopy. A benchtop spectrophotometer was used, the Antaris II FT-NIR Analyzer (Thermo Fisher Scientific, Waltham, MA USA). It operates in the 1000 to 2500 nm (10000−4000 cm^{-1}) range with an average spectral resolution of 1 nm. The spectra were measured in diffuse reflectance mode, avoiding any sample preparation. The built-in viscous liquid sampler, used as a clamp to apply constant pressure and keep the leaf flat, was placed on top of the leaf to standardize the measurement (Figure S1). The final spectrum was obtained by averaging 25 scans, with a blank reference measured once per sample.

2.5. Midinfrared Spectroscopy. A portable spectrophotometer, the Cary 630 ATR-FT-MIR spectrometer (Agilent, Santa Clara, CA, USA) was used, equipped with a 2 mm^2 single bounce diamond crystal sample plate. When scanning the samples, 32 sample scans were collected with a wavenumber range of 4000−850 cm^{-1} (2500−11764 nm) and an average resolution of 2 cm^{-1}. A background spectrum was recorded using air at the beginning of the measurements and once every 15 min. The leaf samples were pressed onto the diamond crystals using a pressure clamp with a slip clutch press to ensure consistent pressure during measurement (Figure S1).

2.6. Spectral Data and Preprocessing Strategies. Spectra were imported to MATLAB (R2023a, 9.14; MathWorks, Natick, MA, USA) and multivariate analysis was performed in MATLAB and PLS_Toolbox (v9.3; eigenvector Research Inc., Earglerock, CA, USA). The general spectral data matrices used for ASCA and PLS models consisted of 704 × 1664 for MIR and 704 × 1557 for NIR, where 704 corresponds to the total number of samples: 32 leaf samples × 2 sides × 11 weeks. Different subsets of matrixes were used for different models and indicated in each section. For each sample and leaf side, spectra were acquired in two instrumental replicates, which were averaged. Each condition in the experimental design included four biological replicates, which were maintained as independent observations throughout both ASCA and PLS modeling. In ASCA, the objective was to assess the effect of experimental factors on spectral variance; thus, no preprocessing was applied to preserve the structure of variability in the original data matrix (X).

To optimize model performance and ensure comparability across spectral techniques, various preprocessing strategies were evaluated using the ProSpecTool toolbox, which systematically explores and ranks preprocessing pipelines based on their impact on predictive quality and model robustness. The preprocessing techniques tested included Standard Normal Variate (SNV), a scatter-correction method that standardizes each spectrum by centring it to zero mean and scaling it to unit variance. First-order linear detrending was also applied to eliminate baseline shifts or linear trends across the spectral range. Savitzky-Golay (SG) smoothing was employed to reduce spectral noise; this method fits a polynomial of defined order (O) within a moving spectral window (W). Gaussian filtering applies a smooth weighted average across the spectrum. These preprocessing methods were applied either individually or in combination, depending on the property being predicted and spectral region under study.

To evaluate whether both leaf sides provide complementary information, a low-level data fusion (LL-DF) strategy was implemented. This approach consists of concatenating the preprocessed spectra from the adaxial (upper) and abaxial (lower) leaf sides into a single feature vector prior to modeling. By combining the two spectral profiles at the raw data level, this strategy allows the model to exploit side-specific physiological differences.

https://doi.org/10.1021/acsagscitech.4c00760
ACS Agric. Sci. Technol. XXXX, XXX, XXX−XXX

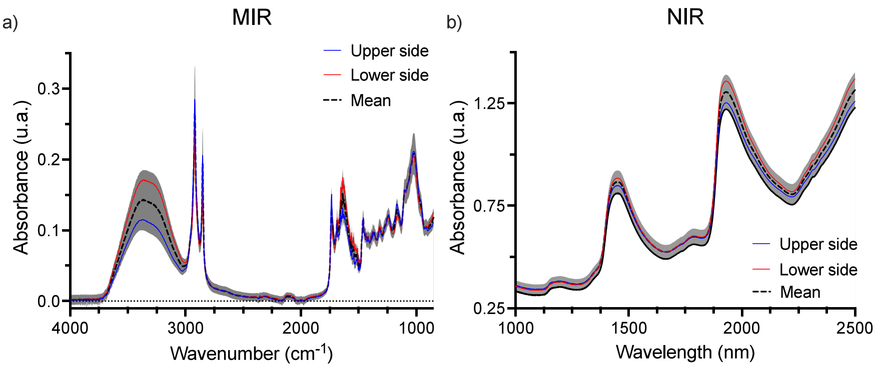

Figure 1. Raw spectra of nectarine leaves obtained using a) a mid-infrared spectrometer and b) a near-infrared spectrometer. The gray-shaded area represents the variability observed in the spectra of nectarine leaves. The dotted line indicates the mean spectrum of all leaves. The blue line shows the mean spectrum for the upper side, while the red line represents the mean spectrum for the lower side.

2.7. ANOVA – Simultaneous Component Analysis.

ANOVA-simultaneous component analysis (ASCA) is a statistical technique used to analyze complex data sets in which multiple factors may simultaneously influence the observed data. This technique is particularly useful in experimental designs where multiple factors are manipulated at once aiming to determine the relative contributions of each factor to the observed variation in the data.

ASCA combines ANalysis Of VAriance (ANOVA) and Simultaneous Component Analysis (SCA) to identify and quantify significant sources of variation in the data. The method involves decomposing the original data matrix into different factor matrices based on the Design of Experiment (DoE) and the explained variance by each factor is then quantified using an ANOVA approach. Finally, the resulting matrices are analyzed using SCA (a similar approach to Principal Component Analysis, PCA) to identify the underlying patterns of variation within the data. Therefore, the ASCA model decomposed the original data matrix (**X**) as

$$\begin{aligned}\mathbf{X} = {} & \mathbf{X}_{\text{Week}} + \mathbf{X}_{\text{Orientation}} + \mathbf{X}_{\text{Height}} + \mathbf{X}_{\text{Prominence}} + \mathbf{X}_{\text{Side}} \\ & + \mathbf{X}_{\text{Week x Orientation}} + ...\mathbf{X}_{\text{Week x Height}} + \mathbf{X}_{\text{Week x Prominence}} \\ & + \mathbf{X}_{\text{Week x Side}} + \mathbf{X}_{\text{Orientation x Height}} + \mathbf{X}_{\text{Orientation x Prominence}} \\ & + ...\mathbf{X}_{\text{Orientation x Side}} + \mathbf{X}_{\text{Height x Prominence}} + \mathbf{X}_{\text{Height x Side}} \\ & + \mathbf{X}_{\text{Prominence x Side}} + \mathbf{E} \end{aligned} \quad (1)$$

where **X** is the original data matrix, $\mathbf{X}_{\text{Factor}}$ is the matrix representing the effect of a Factor in the original data, $\mathbf{X}_{\text{Factor x Factor'}}$ is the matrix representing the effect of the binary interaction of Factor and Factor' in the original data and **E** is the residual matrix.[15]

ASCA offers several advantages over traditional methods for analyzing complex data sets. First, it independently identifies significant sources of variation in the data, even when individual factors have weak effects. Second, it can detect interactions between factors that may be overlooked by other methods. Finally, ASCA presents the results in an easily interpretable visual format. To assess the statistical significance of the results, a permutation test with 1000 iterations was performed.

2.8. Partial Least-Squares Regression.

The prediction of nectarine attributes from leaf spectra was done using the Partial Least Squares Regression (PLSR) algorithm. PLSR modeling is based on projecting the spectra and physicochemical properties into a new space where the covariance between them is maximized, thus simplifying the complexity of the data while retaining the most important information. The ProSpecTool toolbox[16] was used to select the adequate spectral preprocessing method for each instrument and each property, and the selection of the optimal preprocessing and number of latent variables was performed based on the minimum value of the J-Score,[17] which takes into account the performance, the overfitting and the robustness.

Model performance was assessed using both cross-validation and external validation strategies. Cross-validation was performed using a 5-fold random split, repeated 10 times, to ensure stability and reduce variability in RMSECV estimation. Additionally, each PLS data set was independently subjected to external validation using the XYOnion algorithm, a layer-based method that considers distances in both predictor (X) and response (Y) spaces to generate balanced and representative calibration and validation subsets.[18] This approach avoids extrapolation and ensures that validation subsets reflect the full data space variability, thus providing more realistic and reliable model performance estimates. As this splitting is specific to both the X and Y blocks, it was applied separately for each property. The splitting proportions were 60% for calibration and 40% for validation.

The performance of the PLSR models was assessed using several parameters: the coefficient of determination (R^2), the root-mean-square error of cross-validation ($RMSE_{CV}$) and of prediction ($RMSE_{Pred}$), the ratio of performance to deviation (RPD), and the range error ratio (RER). RPD, calculated as the ratio of the standard deviation of the target variable to RMSEP, evaluates predictive ability of the model. Typically, an RPD between 1.5 and 2.0 indicates fair predictive ability and >2.0 is considered good. RER is the ratio of the range of the target variable to RMSECV. Values between 5 and 10 suggest moderate prediction capability, while values above 10 indicate strong predictive performance.

$$R^2 = 1 - \frac{\sum_{i=1}^{N} (y_i - \hat{y}_i)^2}{\sum_{i=1}^{N} (y_i - \overline{y})^2} \quad (2)$$

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{N} (y_i - \hat{y}_i)^2}{N - 1}} \quad (3)$$

where y_i is the observed value, $\hat{y}_i$ is the predicted value, $\overline{y}$ is the mean of observed values, and N is the number of samples.

$$\text{RPD} = \frac{s_y}{\text{RMSEP}} \quad (4)$$

where s_y is the standard deviation of the reference values in the validation set.

$$\text{RER} = \frac{y_{\max} - y_{\min}}{\text{RMSEP}} \quad (5)$$

where $y_{\max}$ and $y_{\min}$ are the maximum and minimum reference values in the validation set.

3. RESULTS AND DISCUSSION

3.1. Spectral Properties.

Each leaf was analyzed first using the NIR spectrometer and then the MIR spectrometer, since the MIR requires the use of the built-in press of the spectrometer, which leaves a mark on the leaf. Every leaf was analyzed with both instruments on the two sides and at two different points on each side. A representation of the spectral variability is shown in Figure 1.

The MIR spectra (Figure 1a) exhibit absorbance peaks in regions typically associated with organic compounds found in plant tissues, such as carbohydrates, lipids, proteins, and other key biomolecules. Peaks around 3000 cm^{-1} are commonly attributed to C−H stretching vibrations, indicative of lipid content, while those near 1000 cm^{-1} likely correspond to polysaccharide structures and other carbohydrate-related functionalities. In addition, smaller peaks in the region between 1500−1700 cm^{-1} could be linked to amide I (C═O bond) and amide II (N−H bond) bands, typically associated with proteins and their secondary structures. These findings align with known spectral patterns in plants and demonstrate the utility of MIR for identifying functional groups relevant to leaf physiology.[19,20]

While both sides of the leaf exhibit similar spectral characteristics, there are differences in peak intensities. For example, the peak around 2920 cm^{-1} (associated with C−H stretching) is slightly more intense on the upper side which may indicate a variation in the lipid components on this surface. Similarly, the region near 1000 cm^{-1} shows a slight divergence, potentially indicating variations in carbohydrate composition or structure between the two sides of the leaf. Although subtle, these differences likely reflect localized metabolic processes or structural distinctions across the leaf surfaces.

In the NIR spectra (Figure 1b), the absorbance peaks around 1200, 1450, and 1900 nm correspond to water absorption bands and overtones of O−H and C−H stretching vibrations. Peaks at around 1200 nm and 2100 − 2300 nm may also represent combinations of C−H bonds related to waxes or cellulose, which contribute to the physical structure and protective layer of the leaf.[19,21]

The consistency in the NIR spectra between the two sides suggests homogeneity in water content and structural composition across both. However, subtle differences in the intensity of the water absorption peaks (particularly at 1450 nm) could indicate slight variations in water retention capacity between the two surfaces, potentially influenced by environmental factors such as light exposure or air circulation.[22]

3.2. Determination of Factors Affecting Analysis of Leaves. To determine the contribution of each considered sampling factor to the evolution of leaves throughout the ripening period, ASCA was applied to the sample-wise unfolded matrix. The original data set consisted of 32 samples, measured on two sides, across 1664 spectral variables (MIR) or 1557 variables (NIR), over 11 weeks (i.e., 32 × 2 x 1664/1557 × 11), which was unfolded into 704 × 1664 (MIR) and 704 × 1557 (NIR) matrices. These matrices included all measured spectra, with each row representing a single sample, and the different sampling days arranged sequentially. The corresponding Design of Experiment (DoE) matrix encoded each factor in a column as 'Week' (1 to 11), 'Orientation' (SE or NW), 'Height' (middle-height or top of the tree), 'Prominence' (near the trunk or exterior), and 'Side' (upper, adaxial, and lower, abaxial, sides). The results for the ASCA models of leaves analyzed with MIR and NIR spectroscopy are summarized in supplementary Table S1.

These results indicate that the 'Week' and 'Side' factors are the primary factors influencing spectral variability in the leaves proximal to nectarines in both the MIR and NIR regions. Specifically, 'Week' accounts for around 15% of the variation in the MIR spectra and 18% in the NIR spectra, while 'Side' contributes around 32% and 16% in the MIR and NIR regions, respectively. In both techniques 'Week' and 'Side' are among the main contributors to spectral variability. In the MIR region, 'Side' explains more variance than 'Week', while in the NIR region the opposite is true, with 'Week' contributing slightly more than 'Side'. These differences may be related to the greater sensitivity of the MIR fingerprint region to surface and compositional differences between leaf sides.[23]

The significance of the 'Side' factor indicates that the leaf surface plays a crucial role in the observed spectral variations.[24] The differences between the two sides could be attributed to variations in photosynthetic activity, leaf temperature over time, differences in the cuticular layer, the wax layer that protects plant tissues, and other metabolites that are known to vary depending on the leaf side and the amount of sunlight received by different parts of the plant.[25,26]

Since the influence of the leaf side (Table S1), as expected, is substantial enough to mask the effects of the rest of the factors, the matrices were split to study each leaf side individually. Consequently, four ASCA models were built: one for each instrument on each leaf side, with data matrices of 352 × 1664 and 352 × 1557 for MIR and NIR, respectively. The results of all ASCA models are summarized in Table 1. ASCA loadings corresponding to the significant effects reported in Table 1 (p-value <0.05) are shown in Figure S2.

Table 1. Results of the ASCA Applied to the Sample-Wised Unfolded Matrixes (Mid-Infrared (MIR) and Near-Infrared (NIR) Spectra) Divided between Both Sides of Leaves, Considering the Main Factor from the DoE, Except "Side" and Their Two-Way Interactions

	Effect (%), MIR		Effect (%), NIR	
Factor	Upper Side[a]	Lower Side[b]	Upper Side[a]	Lower Side[b]
Week	25.23[c]	27.00[c]	28.93[c]	22.11[c]
Orientation	0.06	0.23	0.86[c]	0.66
Height	0.20	0.08	0.54	0.38
Prominence	0.57	0.14	0.28	0.11
Week × Orientation	1.12	1.52	2.98	3.93[c]
Week × Height	2.16	1.85	1.17	1.89
Week × Prominence	1.47	2.48	2.70	3.81[c]
Orientation × Height	0.27	0.54	0.08	0.27
Orientation × Prominence	0.12	0.30	0.53	0.15
Height × Prominence	0.26	0.32	0.03	0.32
Residuals	68.54	65.54	61.90	66.38

[a] Upper side of the leaf, adaxial. [b] Lower side of the leaf, abaxial. [c] Significant factor (p-value <0.05).

The ASCA model results reveal that 'Week' is the most significant factor for explaining spectral variation across both the upper (adaxial) and lower (abaxial) sides of nectarine leaves in the MIR and NIR regions. In the MIR region, 'Week' explains around 25% and 27% of the variation for the upper and lower side, respectively, while in the NIR region it accounts for 29% and 22%. The significant influence of the 'Week' factor likely reflects the physiological changes in the leaves as the nectarines ripen. As the fruit develops, it induces alterations in the surrounding leaves' metabolic activity, which in turn affects their spectral features. These changes may

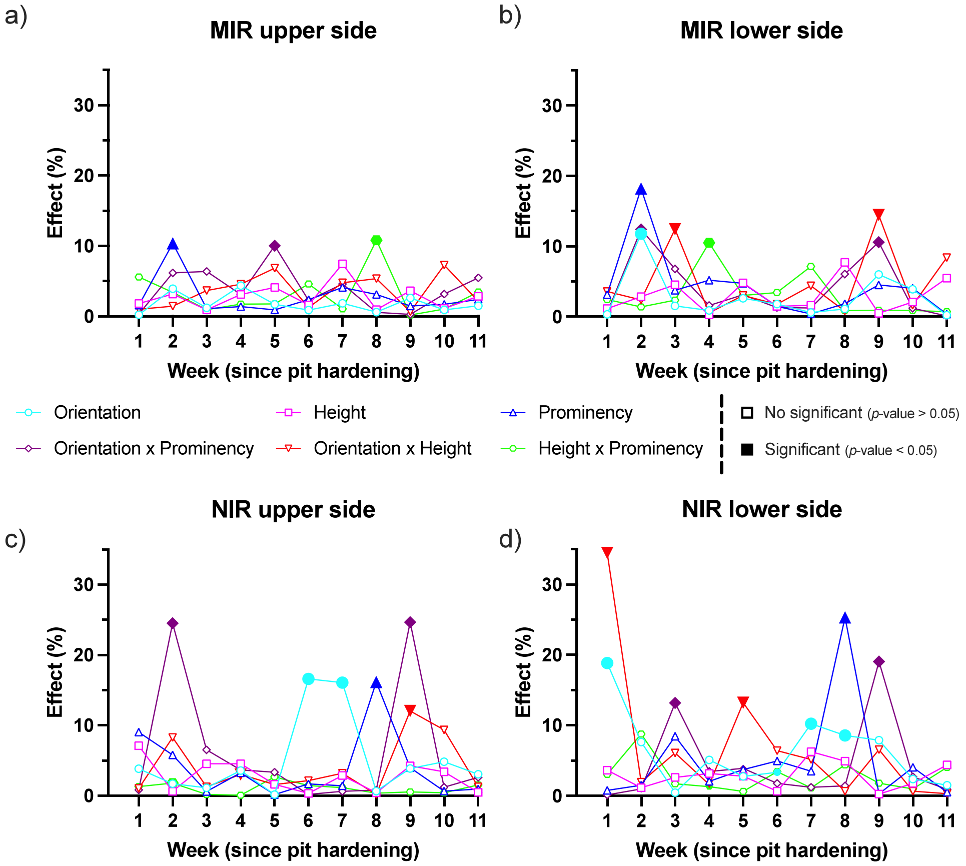

Figure 2. Evolution of factors affecting the spectra of leaves for each instrument (a,b for mid-infrared (MIR) spectroscopy and c,d for near-infrared (NIR) spectroscopy) for each of the sides of the leave (a,c for the upper part (adaxial), and b,d for the lower part (abaxial)) during the different sampling days, where significant factors (p-value <0.05) are represented as filled points.

include variations in chlorophyll content, water status, or the accumulation of secondary metabolites.[27] The influence of water status on leaf spectra has been widely explored in literature[7,28,29] and the proposed methodology accounts for water content evolution over time that may be related to the ripening dynamics. Additionally, since this spectroscopic analysis is intended for direct field measurements, the leaves were analyzed without drying or grinding to allow for in situ measurements.

This is further explained, based on Figure S1, as in the MIR region, the stronger influence of the "Week" factor appears to reflect chemical and structural differences between the upper and lower surfaces of the leaf, particularly in regions around 2920 cm^{-1} and 1000−1200 cm^{-1}, which are typically associated with lipid and polysaccharide content. In contrast, the "Week" factor in the NIR region has sensitivity to bulk compositional changes, especially water content and metabolite evolution throughout the ripening process. This is supported by the presence of strong absorption bands near 1450 and 1940 nm, corresponding to the first and second overtones of O−H stretching vibrations, which are directly related to water status, as well as regions around 1200−1250 nm and 2100−2300 nm, linked to C−H and N−H combinations from carbohydrates and proteins.

The great effect of the 'Week' factor appears to mask the effects of other experimental factors. For example, the individual effects of 'Orientation', 'Height', and 'Prominence' factors on spectral variability are minimal and nonsignificant in both MIR and NIR spectra. Although 'Orientation' shows a weak but significant effect in the NIR region (below 1% for the upper side), it remains relatively minor compared to the high influence of 'Week'. This suggests that the orientation of the leaf (SE or NW) interacts with temporal changes, possibly reflecting different light exposure dynamics as the growing season progresses. However, the changes driven by time are far more pronounced.

Several interactions involving 'Week' are also noteworthy. The interaction between 'Week' × 'Orientation' is significant in the NIR region for the lower side, accounting 4% to the variation. Similarly, the interaction 'Week' × 'Prominence' is significant in both the MIR and NIR regions, contributing around 4% of the variation on the lower side. These interactions suggest that temporal changes influence how leaf position within the tree canopy and its exposure to sunlight affect spectral features.

Other factors, such as 'Height' and 'Prominence', showed minimal and generally nonsignificant effects on their own. The individual contributions of these factors were always below 1%, indicating their limited influence on overall spectral variation.

When comparing these results with those describing the variation in nectarine fruits, obtained by Ezenarro et al.,[14] the influence of the global position, when measured using spectroscopy, is greater in nectarines than in leaves. This difference is likely due to the role nectarines play as a 'reservoir' for metabolites generated and transported from the plant, whereas leaves possess a self-regulatory capacity that allows them to respond dynamically to stimuli or stressors, making them inherently more challenging to study.

Table 2. Partial Least Square Regression Results and Figures of Merit for Mid-Infrared (MIR) and Near-Infrared (NIR) Spectra of Models with the Best Performance Parameters for Each Property

Property	Region	Matrix	Preprocessing	# LV[a]	R^2_{CV}[b]	$RMSE_{CV}$[c]	R^2_P	$RMSE_P$	RPD[d]	RER[e]	J-Score[f]
Weight (g)	MIR	LL-DF[g]	Gaussian W11 SNV	9	0.637	40.52	0.674	39.74	1.75	6.06	0.241
	NIR	LL-DF	Gaussian W15 Detrending	10	0.637	41.99	0.615	40.23	1.59	5.46	0.233
Firmness (kg)	MIR	LL-DF	Gaussian W9 Detrending	9	0.729	1.0	0.667	1.1	1.71	7.19	0.237
	NIR	LL-DF	SG W13O2 SNV	8	0.527	1.5	0.568	1.5	1.45	7.68	0.259
TSS (°Brix)	MIR	LL-DF	SG W11O2 SNV	8	0.463	1.9	0.629	1.8	1.64	6.78	0.270
	NIR	LL-DF	Gaussian W15 SNV	9	0.593	1.8	0.645	1.6	1.67	7.33	0.243
pH	MIR	LL-DF	Gaussian W9 SNV	9	0.553	0.18	0.617	0.16	1.62	5.34	0.256
	NIR	2[h]	SG W15O2 Detrending	9	0.433	0.20	0.571	0.17	1.50	5.18	0.286
Acidity (malic acid eq g · L^{-1})	MIR	1[i]	SG W3O2D1 SNV	2	0.312	0.48	0.416	0.49	1.30	5.41	0.384
	NIR	Mean[j]	SG W15O2 SNV	4	0.273	0.56	0.306	0.56	1.18	5.13	0.354

[a] Number of Latent Variables selected for the model. [b] Determination coefficient in cross-validation (CV) or prediction (P). [c] Root Mean Square Error of Cross-Validation (CV) or prediction (P). [d] Ratio of Performance to Deviation. [e] Range Error Ratio. [f] Joint-score based on Ezenarro et al. (2023). [g] Low-level data fused spectra of both leaf sides by concatenation. [h] Spectra of the upper (adaxial) side of the leaf. [i] Spectra of the lower (abaxial) side of the leaf. [j] Average spectra between both leaf sides.

Finally, a considerable portion of the variation remains unexplained by the model, with residuals accounting for up to 69%. This suggests that additional factors not captured in the experimental design, along with the inherent variability within the samples, may contribute to the observed spectral differences in the leaves proximal to the ripening nectarines. Considering that leaves were collected from a real orchard, this result is to be expected as local weather conditions, possible stresses caused by biotic and abiotic factors, and the inherent metabolic variability of the tree, can all influence the ASCA results.[30,31] Nevertheless, the similarity in residual values across all four data sets indicates a consistent pattern of unexplained variability, which could reflect the complexity of studying plant systems in natural, dynamic environments and the leaves' physiological response to both internal metabolic processes and external factors.

3.3. Week-Wise Stratification Variability Study. Given the significant influence of the 'Week' factor and its interactions with other factors highlighted in previous ASCA models, this factor was isolated for each instrument and leaf side to allow for a more detailed analysis. The primary objective of this work is to understand how each positional factor affects the ripening process in leaves, which could not be effectively interpreted in the ASCA model due to the dominant effect of the 'Week' factor. Therefore, 22 ASCA models were built for each instrument, using data matrices of 32 × 1664 and 32 × 1557 variables for MIR and NIR, respectively. The results of these ASCA models are summarized in Figure 2.

The results of all the ASCA models confirmed that the influence of the considered factors is dependent on the stage of the ripening cycle of the nectarines, as expected. For the MIR data (Figures 2a and 2b), factor effects significantly vary over time, with significance at specific weeks, especially around weeks 8 and 9. These weeks are particularly relevant, as they correspond to the harvest period as determined by the orchard owner based on overall fruit maturity. On the sun-exposed side (MIR upper side), orientation and its interactions show considerable effects early in the season (weeks 1−2) and again near harvest, with some factors reaching up to 20% of the variance. The opposite side (MIR lower side) shows similar trends but with fewer significant factors, highlighting that the response of nectarine leaves to these factors is not symmetric between the two leaf sides. These findings suggest a specific response of the leaves to environmental conditions, such as sunlight exposure and orientation and demonstrate that the ASCA approach is an effective tool for quantifying those effects at each ripening stage.

In the NIR spectra (Figures 2c and 2d), the variability of factor effects is even more pronounced, with some factors, such as the interaction between orientation and prominence, showing significant effects over 25% around weeks 3 and 9. Interestingly, for both sides of the leaf, different factors affect at different times, reflecting the dynamic physiological processes in the leaves as they mature; this suggests a relationship between leaf development stages and the corresponding changes in spectral properties with maturation.

When compared with the findings of Ezenarro et al.[14] in the study on nectarines, a noteworthy relation is found as several factors that show significance in the leaf spectra are later reflected in the fruit spectra at more advanced stages of development. For instance, in NIR spectra of leaves, there is a significance of the factor 'Orientation' for weeks 6 and 7 (upper side) and 7 and 8 (lower side), which then it can be seen for nectarines in weeks 7 to 9. Another example is the 'Prominence' factor, which is significant for leaves at week 8 for both sides, and then it is significant for nectarines at week 9. This suggests that the changes detected in the spectral characteristics of the leaves act as early indicators of the physiological processes that will later become evident in the fruit. This delayed correspondence highlights the dynamic interaction between leaf and fruit physiology, wherein the leaves respond to environmental or internal stimuli, and these changes are gradually communicated or manifested in the fruit over time.[32] These results indicate that noninvasive measurements taken from leaves can serve as reliable early predictors of fruit properties.

Moreover, the asymmetry observed between the different sides of the leaf and the differences found between the two spectroscopic methods (MIR and NIR) suggest that the spectral characteristics of the leaves are influenced by microenvironmental factors and by leaf-specific morphological traits. These variations could be critical when using spectroscopy for noninvasive monitoring of nectarine leaf and fruit relationships.[33] Previous studies on nectarines have similarly shown that factors such as orientation, exposure to sunlight, and sampling time can influence spectral data.[14,34] The current findings reinforce this understanding, adding that these factors do not act independently but through complex interactions, which can vary over time. Furthermore, the significant factors observed at or near harvest indicate that these temporal changes in spectral properties should be considered to improve the accuracy of maturity predictions in nectarine fruits.

3.4. Prediction of Nectarine Properties. Nectarine samples collected alongside their proximal leaf were also analyzed using the classical approach for nectarine ripeness assessment, in terms of weight, firmness, TSS, pH and total acidity. In line with the goal of using leaves as indicators of nectarine ripening, this work also aimed to predict fruit properties based on leaf spectra. To achieve this, different PLS regression models were built using different spectra matrices: each side individually, the average spectra from both sides and a low-level data fusion (LL-DF) approach by concatenating the spectra of both sides. Model performance parameters were calculated to compare all models (Tables S2 and S3) and the models with the best performance are summarized in Table 2.

The PLS regression results for predicting nectarine properties using MIR spectra of leaves (Table S2) indicate that no single preprocessing method or matrix configuration yielded universally optimal results across all parameters. Model effectiveness varies with specific nectarine attributes, with weight and firmness showing the most accurate predictions. To assess the actual benefit of preprocessing, models were also tested using raw spectra. In all cases, preprocessing improved model performance, both in terms of predictive accuracy and robustness. The raw spectra consistently resulted in lower R^2 values and higher $RMSE_P$.

For weight, the LL-DF model achieved the highest R^2_P of 0.674, a relatively low $RMSE_P$ of 40 g, an RPD of 1.75, and a RER of 6.06 indicating a useful model with moderate predictive power, since RPD and RER thresholds to be considered good models are 2 and 10, respectively. Models using mean and individual leaf sides spectra showed slightly lower, but still acceptable, performance. The higher accuracy in weight prediction can be attributed to a strong correlation between leaf physiological status (captured by MIR spectra) and the overall fruit biomass, which has been already observed in other studies of leaf-to-fruit interaction.[35]

Firmness prediction models were similarly successful, with the LL-DF model showing the best performance ($R^2_P = 0.667$, $RMSE_P = 1.1$). This model has a greater performance than the models with all the other matrix arrangements, including the models based on single-side spectra. Firmness, being a mechanical property, is closely related to the fruit's maturity and the physiological changes occurring in the proximal leaves, explaining the strong predictive power of these models.

TSS and pH models were moderately accurate and provided useful predictions with the LL-DF model, performing best for TSS (R^2_P of 0.629 and an RPD of 1.64) and for pH (R^2_{CV} of 0.617 and an $RMSE_P$ of 0.16). These results suggest that the spectral data of the proximal leaf capture relevant information related to the sugar accumulation but do not entirely capture the subtle changes in fruit acidity, so pH correlations were weaker.

Total acidity models, however, showed poor predictive capability, with the best model (upper side spectra) achieving a relatively low R^2_P of 0.416. This limited success in these models can likely be attributed to the fact that total acidity is not ~~as~~ strongly related to the physiological properties of the proximal leaves.

The results demonstrate that LL-DF consistently outperformed single-side spectra models, highlighting the importance of incorporating spectral data from both sides of the nectarine proximal leaf to improve predictive accuracy. These findings confirm ASCA results showing that each side contains distinct information, and that combining them provides complementary insights that enhance model performance. Previous findings by Tardaguila et al. have similarly demonstrated that spectral data from different leaf sides have different predictive performances.[36] By capturing a more complete physiological profile of the leaf, the LL-DF approach better reflects the relationship between leaf spectra and fruit properties. Its better performance in predicting weight and firmness suggests that this method can be particularly useful for parameters closely associated with fruit structure and maturity. While the overall performance for some parameters, particularly total acidity, was limited, it is important to note that the spectral data used in these models were obtained from proximal leaves rather than the fruit itself. This also reinforces the value of acquiring spectral information from both leaf sides. These results highlight the potential of leaf mid-infrared spectroscopy as a noninvasive tool for estimating specific fruit properties, though further models and method improvements may be necessary for more accurate predictions.

The PLS regression results for predicting nectarine properties using NIR spectra (Table S3) similarly demonstrate a range of model performances depending on the parameter being predicted and the matrix configurations. Similar to the MIR spectra, weight and firmness were among the best-predicted parameters using NIR. The LL-DF model for weight achieved the highest R^2_P of 0.615, a low $RMSE_P$ of around 40 g, an RPD of 1.59, and an RER of 5,46, marking this model as moderately predictive. For firmness, the LL-DF model also showed the best performance, with an R^2_P of 0.568 and an $RMSE_{CV}$ of 1.5, along with an RPD of 1.67, highlighting its predictive capability. Although firmness predictions were less accurate than weight, the LL-DF approach consistently outperformed single-side models which exhibited lower R^2 values. For TSS, the LL-DF model was again among the best ($R^2_P = 0.645$, RPD = 1.67), indicating moderate predictive capability. This finding aligns with the MIR results, indicating that the combination of the spectral data from both sides of the leaf improves the prediction of sugar content in the fruit.

For pH models, the model for the lower leaf side yielded an R^2_P of 0.571 and an $RMSE_P$ of 0.17, indicating modest predictive power. The total acidity models were the most challenging, with low R^2 values across all model configurations. The mean model performed particularly poorly for this parameter ($R^2_P = 0.306$, RPD = 1.18), underscoring the difficulty of using NIR spectra to predict total acidity.

Overall, these results show the potential of using NIR leaf spectra, particularly through LL-DF, as a noninvasive tool for estimating certain nectarine properties. Combining spectral data from both sides of the nectarine leaf enhances the physiological profile of the leaf, improving the prediction of fruit properties. However, further refinement of the models, particularly for predicting biochemical properties like acidity, may be needed for greater accuracy of these predictions.

When comparing MIR and NIR for predicting nectarine properties from leaf spectra, distinct trends are observed. MIR spectroscopy generally offered slightly better predictions for weight and firmness, with higher R^2 values and lower RMSECV values, whereas NIR performed comparably on

TSS and pH, even slightly surpassing MIR in RPD for TSS. Both MIR and NIR struggled to accurately predict total acidity, achieving low R^2 and RPD values in all models. Data fusion strategies between both spectroscopic regions were not attempted to keep the analytical approach fast and simple, without using more than one instrument. This comparison highlights that while both techniques are valuable for noninvasive fruit property predictions, in complex biological process, such as fruit ripening. MIR tends to provide slightly better accuracy for mechanical properties like weight and firmness, whereas NIR may capture biochemical variations in the fruit more effectively, particularly for TSS.

This study demonstrates the potential of using leaves, specifically their NIR and MIR spectra, as a nondestructive approach to monitor nectarine ripening. The results revealed spectral differences between the upper (adaxial) and lower (abaxial) leaf sides, each showing unique spectral patterns and intensity variations in both MIR and NIR regions. These results reflect differences in composition and interaction with both techniques in both sides, as the two sides of the leaf respond to environmental and metabolic cues.

ASCA models identified the most influential factors on leaf spectral variability, highlighting 'Week' as the dominant contributor in both MIR and NIR spectra. This factor is reflective of temporal physiological changes, emphasizing their importance in ripening prediction models. The week-wise unfolding of data in this study allowed capturing these temporal metabolic shifts, showing how leaf position influences spectral responses and offering an enriched view of ripening dynamics. This detailed tracking, though complex, offers a novel, time-based understanding of leaf and fruit interplay. Future work should integrate direct measurements of leaf moisture and targeted biochemical markers alongside spectroscopic data to more quantitatively disentangle the contributions of water status and leaf metabolites, thereby strengthening the mechanistic interpretation of this relationship.

The predictive performance of PLS regression models in this study further reinforces the promise of leaf spectroscopy as a precision agriculture tool. The PLS regression models to predict fruit properties like weight and firmness further support the validity of leaf spectroscopy as a reliable tool. While the models for acidity and pH exhibited lower predictive power, the overall findings suggest that fusing spectral data from both sides of the leaf enhances model accuracy. The limitations observed, particularly in predicting biochemical properties, underscore the need for further refinement of the approach and consideration of additional factors influencing leaf physiology. However, one main advantage is the possibility of assessing fruit maturity by leaf analysis on-site or in the laboratory without the need for fruit destruction or quality decrease.

In conclusion, the spectroscopic analysis of leaves offers a promising, noninvasive methodology for assessing nectarine quality, potentially transforming current fruit monitoring practices. This approach, with continued development, could provide a valuable tool for precision agriculture and real-time decision-making in horticulture.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsagscitech.4c00760.

Table S1. ASCA results for MIR and NIR spectra with main factors and two-way interactions. **Table S2**. PLS regression results and figures of merit for MIR spectra. **Table S3**. PLS regression results and figures of merit for NIR spectra. **Figure S1**. Graphical representation of leaf spectra collection. **Figure S2**. ASCA loadings of significant factors (Week, Week × Orientation, Week × Prominence) (PDF)

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Funding

Grants PID2019-104269RR-C33 funded by MICIU/AEI/10.13039/501100011033. Grants URV Martí i Franqués − Banco Santander (2021PMF-BS-12; Ezenarro, J.) and URV Martí i Franqués − IRTA (2020PMF−PIPF-6; García-Pizarro, Á).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors would like to thank Ricard Torrent Pellisé, the helpful owner of the nectarine orchard and the technicians of the UPC and IRTA who helped with the sample collection and measurements. During the preparation of this work the authors used ChatGPT 4o in order to edit text and improve readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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