Non-destructive assessment of 'Fino' lemon quality through ripening using NIRS and chemometric analysis

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Abstract

The lemon industry has the challenge of providing fruits with high-quality standards worldwide. Replacing the subjective fruit quality assessment methods with objective and non-destructive techniques. Total soluble solids (TSS) and titratable acidity (TA) have been revealed as important ripening markers in lemons. Therefore, this study proposes, for the first time, using near-infrared spectroscopy (NIRS) as a rapid and non-destructive alternative to evaluate these quality traits in 'Fino' lemons (*Citrus limon* L. Burm) during ripeness. NIR spectra (950-1700 nm) of intact lemons collected from two different orchards at three ripening stages were acquired, while standard destructive methods were used to determine TSS and TA in the juice of each fruit. The prediction of the quality parameters was carried out using partial least squares regression (PLS-R) models. Three approaches were followed to validate the models: internal, external, and recalibrated external validation. The results following the first approach presented a good predictive performance for both quality parameters (TSS: R^2 = 0.84, RMSEP = 0.42 and RPD = 2.5; TA: R^2= 0.72, RMSEP = 0.45 and RPD = 2.0). When the external validation was performed, the best results were obtained for the TSS prediction using recalibrated models, maintaining good predictive performance accuracy (R^2 = 0.74 and 0.67, RMSEP = 0.42 and 0.58, and RPD = 2.4 and 1.7). Regarding distinguishing different origins, models based on partial least squares discriminant analysis (PLS-DA) were externally validated, achieving 66.4 % correct classification, respectively. Thus, applying NIR technology in the lemon fruit packinghouses is a promising alternative to improve fruit management and meet consumer demands.

Keywords: citrus; quality; non-destructive; spectroscopy; chemometrics

Abbreviations

CV: cross-validation

LV: latent variables

MSC: multiplicative scatter correction

NIR: near-infrared

NIRS: NIR spectroscopy

SEL: standard error of the laboratory

PC: principal component

PCA: principal component analysis

PLS: partial least squares

PLS-R: PLS regression

PLS-DA: PLS discriminant analysis

RMSE: root mean square error

RPD: residual prediction deviation

R^2: coefficient of correlation

TA: titratable acidity

TSS: total soluble solids

1. Introduction

Citrus is one of the most important crop worldwide. The demand for citrus fruit has increased significantly over the last decade, resulting in a substantial increase in total production from 134.3 million tons to 166.3 million tons (FAO, 2022). Among citrus, lemons (*Citrus limon* L. Burm) rank third in importance, with an estimated production of 21.5 million tons (FAO, 2022). Lemons are highly valued for their sensory characteristics, making fresh consumption the preferred choice among consumers, and it is an essential ingredient in cooking or baking. Furthermore, this fruit is an important source of antioxidant compounds such as ascorbic acid and flavanones, which offer significant health benefits (González-Molina et al., 2010). However, fruit ripeness at harvest is a critical factor determining the quality traits during marketing.

Nowadays, the lemon fruit industry uses external and internal characteristics such as size, colour, absence of physical damage and juice content to assess the fruit quality (EU 428/2019, 2019). However, recent results confirmed the importance of TSS and TA levels for the shelf life of lemons. These results showed that both TSS and TA decrease during the whole harvest season, coinciding with the lowest values with the most ripened fruits (Serna-Escolano et al., 2022. Furthermore, "'Fino' lemons with the highest TSS and TA levels coincided with the highest polyphenolic content and the lowest decay incidence (Serna-Escolano et al., 2023). Strong evidence indicate the importance of carbohydrates and organic acids, maintaining the cell energy requirements and being used as structures to ssynthesise important protectant molecules (Brizzolara et al., 2020). Thus, TSS and TA in lemons are essential for determining ripening at harvest, fruit quality and shelf life.

Traditional destructive techniques to assess fruit quality are time-consuming, require complex equipment and sample preparation, and skilled operators need to perform the analyses. On the contrary, optical non-destructive alternatives for fruit quality monitoring, such as near-infrared spectroscopy (NIRS), have been proven faster, easier, and well-suited for in-line grading and sorting processes (Arendse et al., 2018; Cortés et al., 2019a). Spectroscopy measures how light interacts with a sample. NIR light, typically between 750 and 2500 nm, can be reflected, absorbed, or transmitted through the sample, depending on its chemical composition and physical properties. In fruit samples, these interactions cause vibrations in certain molecular groups, such as carbon-hydrogen (C-H) and oxygen-hydrogen (O-H) bonds, which are mainly related to the water content and storage reserves (Walsh et al., 2020). Yet NIRS relies on indirect measurements that generate complex data and requires the assistance of statistical methods, such as chemometrics, to extract the potential of the acquired spectra (Cortés et al., 2019b). PLS is one of the most used methods to predict quality traits from NIR spectra since this method can deal with strongly collinear, correlated, and noisy data (Wold et al., 2001).

Measuring the internal quality of citrus fruit during ripeness can be challenging due to the interference of NIR light caused by the thickness of the peel (Sun et al., 2020). However, studies conducted on other citrus fruit, such as oranges (Li et al., 2020; Tian et al., 2020; Song et al., 2019; Cavaco et al., 2018; Liu et al., 2015; Sanchez et al., 2013a; Jamshidi et al., 2012; Liu et al., 2012; Cayuela et al., 2008; Zude et al., 2008), mandarins (Pires et al., 2022; Li et al., 2020; Torres et al., 2019; Sanchez et al., 2013b; Antonucci et al., 2011, Xudong et al., 2009; Guthrie et al., 2005ab) and grapefruit (Ncama et al., 2017) showed positive results in prediction of quality parameters such as TSS and TA using NIRS.

In many of these studies, developing prediction models using NIRS involves partitioning the available samples into a calibration and a validation set. However, this internal validation approach does not guarantee success in real-world scenarios, like in-line grading systems, which are dynamic processes (Cavaco et al., 2021). Hence, the validation set should originate from a different dataset, considering factors such as fruit age, orchard, and seasonal variability. In such cases, it is called an external validation set (Walsh et al., 2020).

To date, no study has been carried out on the applicability of NIRS for assessing the quality of lemon during ripening. Thus, the aims of this work were: i) to explore the potential of NIRS in the 950-1700 nm range in predicting the internal quality of 'Fino' lemon fruit concerning TSS and TA content during ripening, ii) to assess the ability of NIRS to differentiate the fruit origin, and iii) to evaluate the robustness of PLS calibration models through both internal and external validations.

2. Material and methods

2.1. Field conditions and experimental design

The experiments were conducted in two commercial orchards of 'Fino' lemon (*Citrus limon* L. Burm), with eight-year-old trees grafted on alemow roostook (*Citrus macrophylla* Wester). The orchard A was located in Librilla, Murcia (Spain) (37°54'11.92" N, 1°20'29.62" W) and the soil had a clay loam texture with 18.6 % clay, 37.6 % silt, and 43.8 % sand, a pH of 8.12, and a conductivity of 0.79 dS/m. The orchard B was in Cartagena, Murcia (Spain) (37°40'22.1" N, 0°50'36.4" W), and the soil had a clay loam texture with 37.6 % clay, 26.2 % silt and 37.2 % sand with a pH of 8.4 and a conductivity of 0.50 dS/m. Both orchards were in a region with typical Mediterranean weather conditions, with an average temperature and rainfall of 18 ºC and 272 mm, respectively. Additionally, both orchards were cultivated using the same standard agronomical practices.

In each orchard, a batch of 70 lemons homogeneous in size (with a diameter not less than 45 mm according to EU 428/2019, 2019) and without external defects were harvested from seven lemon trees (10 fruit per tree) selected at random. The harvests were performed in October (H1), November (H2), and December (H3) of the 2022 season.

Therefore, 420 lemon samples were analysed in this study: 210 from the orchard A and 210 from the orchard B. Although all the lemons had reached commercial maturity, the samples harvested in H1 and H2 showed green and light-green colours, respectively. Therefore, according to the citrus colour index, these lemons were exposed to a degreening process at 25 ºC and 95 % relative humidity to achieve the commercial yellow colour (Cubero et al., 2018). Meanwhile, lemons from the H3 had already reached the commercial yellow colour in the field, so this treatment was unnecessary.

2.2. NIR spectra acquisition and preprocessing

For temperature stabilisation, lemon samples from H1 and H2 were left at room temperature (20 ºC ± 2 °C) for 2 hours after the degreening process and before the acquisition of the spectra. In the case of H3 lemons, they were left at room temperature overnight after collection.

Spectra of all intact lemons were acquired in reflectance mode using a spectrometer (AVS-DESKTOP-USB2, Avantes BV, The Netherlands). The detector (AvaSpec-NIR256–1.7 NIRLine, Avantes BV, The Netherlands) was equipped with a 256-pixel non-cooled Indium Gallium Arsenide sensor (Hamamatsu 92xx, Hamamatsu Photonics

KK, Japan), and a 100 mm entrance slit and a 200 lines/mm diffraction grating. This equipment is sensitive to the NIR range from 950 nm to 1700 nm, with a spectral full width at half maximum resolution of 12 nm. The spectral sampling interval was 3.535 nm. A stabilised 10 W tungsten halogen light source (AvaLight-HAL-S, Avantes BV, The Netherlands) was used. The probe tip was situated at 45° to minimise the specular reflection of the lemon surface.

The system was calibrated using a white reference tile (99 %, WS-2, Avantes BV, The Netherlands). The integration time was set at 150 ms, corresponding to a reflectance of 90 % of the saturation using the white reference. Each spectrum was obtained as the average of ten scans to reduce the detector's thermal noise (Nicolaï et al., 2007).

The measurements were performed at two opposite points in the equatorial part of the intact lemons, averaging both values to obtain a single spectrum for each lemon sample. The mean reflection of each sample (*S*) was converted to reflectance (R) using the white (*W*) and dark (*D*) reference values. The dark reference was obtained by switching off the light source and covering the tip of the reflectance probe. The reflectance values were obtained using Eq (1).

$$R = \frac{S-D}{W-D} \qquad (1)$$

The reflectance (*R*) spectra were transformed to absorbance (A) following Eq (2).

$$A = log\frac{1}{R} \qquad (2)$$

Finally, MSC was applied to the spectra to reduce the possible scattering effects, retaining the most valuable information (Rinnan et al., 2009).

2.3. Determination of internal quality attributes

After the spectral acquisition, TSS and TA were measured in the juice of each lemon sample. TSS were measured with a digital refractometer (Hanna Instruments, Rhode Island, USA), and TA by titration of 0.5 mL of juice with NaOH 0.1 mM until pH 8.1 using an automatic titrator (785 DMP Titrino; Metrohm, Herisau, Switzerland), and results were expressed as % and g of citric acid equivalents per 100 mL^{-1} of juice, respectively (Serna-Escolano et al., 2021).

The effect of each harvest time and the orchard origin on TSS and TA was statistically evaluated. Initially, the data was tested for normal distribution using the Kolmogorov-Smirnov test. Since neither parameter followed a normal distribution, the non-parametric Kruskal-Wallis test was employed to identify significant differences in quality parameters based on harvest time and orchard origin. Subsequently, the post-hoc Dunn test was conducted to identify which groups exhibited significant differences. Significance was established at $p < 0.05$. These analyses were performed using SPSS software, version 22 (IBM Corp., Armonk, NY, USA).

2.4.1 Chemometric analysis

PCA was used to explore differences between samples, detect patterns, and identify essential wavelengths unsupervised. This method reduces the dimensionality of a dataset of cross-correlated variables while retaining most of their variability. To this end, the variables of a dataset are transformed into a new set, called PCs, using linear combinations of the original variables. These combinations are done so that the new variables (PCs) are uncorrelated, and most of the information within the initial variables is retained and compressed into the first components (Jolliffe, 2002). All samples were used to perform this analysis.

The PLS method was used to calibrate the models to discriminate the lemon samples from each orchard and to predict their quality parameters. PLS-R extracts the essential information from the data to build a calibration model that captures the underlying connections between the predictor or spectral data and response or quality properties variables. This is done by transforming these variables into a new latent space where their covariance is maximised (Lorente et al., 2012). As the response variable is categorical, the PLS-DA allows discrimination between groups by maximising the covariance between the predictors or spectral data and the response or orchard origin (Lorente et al., 2012).

Three approaches were followed to build the PLS-R calibration models to predict TSS and TA quality parameters. In the first approach, named internal validation, the data consisting of 420 spectra from lemon samples harvested at different ripening states from orchards A and B was partitioned into two data sets: 70 % of samples (280) were randomly selected to calibrate the models as a training set, and the remaining 30 % (140) were used for test prediction. The second approach, external validation, consisted of validating the models using an independent data set (Walsh et al., 2020). The data from orchard A (210) were selected to calibrate the models, and data from orchard B (210) were used as an independent or external set to validate the models and vice versa. In the third approach, the models of the second approach were recalibrated, adding several external samples to the calibration data set to build a new model. This method, also known as spiking, was previously used in several studies (Pires et al., 2022; Zude et al., 2008; ; Magwaza et al., 2014) for improving the robustness of citrus NIRS models since both groups (training and external validation) had different ranges and it was necessary recalibrate the model to capture all the variability. In this case, 10 % of the samples (21) of the external validation set were randomly selected and included to recalibrate the models.

The internal and external validation approaches were also followed to discriminate the orchard origin. For the first approach, 70 % of the samples (280) were used to calibrate the PLS-DA model, and the remaining 30 % (140) were used for validation. For the second approach, the data were divided into two sets: samples from two harvests (280) were selected to calibrate the models and samples from the remaining harvest (70) were used as an independent or external set to validate the models. Three models were developed using the different combinations of harvests in the calibration and validation sets.

The selection of the optimal number of LVs and the estimation of the error rate of the PLS models were performed using a 10-fold CV on the training set (Hastie et al., 2017). The number of latent variables was determined as that which minimised the RMSE in the CV. The performance of the PLS-R models was evaluated using the R^2 and the RMSE in the calibration (R^2_{CV}, $RMSE_{CV}$) and the validation (R^2_P, $RMSE_P$) sets, respectively. RPD was also calculated as an indicator of the discrimination capability as the ratio of prediction to deviation (Saeys et al., 2005). A model with an RPD value below 1.5 is considered not usable, between 1.5 and 2.0 reveals a possibility to distinguish between high and low values, between 2.0 and 2.5 makes approximate quantitative predictions possible, while a values between 2.5 and 3.0, and above, the prediction is classified as good and excellent, respectively. The results of the PLS-DA model were presented as the percentage of correct classification for both the calibration and validation datasets.

All chemometric analyses were performed using The Unscrambler X 10.3 (CAMO Software, Oslo, Norway).

3. Results and discussion

3.1. Fruit internal quality attributes

Both parameters, TSS and TA, decreased during the fruit growth and ripening on the tree, showing significant differences ($p < 0.05$) between each harvest and orchard origin (Table 1). In this sense, TSS decreased from 8.82 % in H1 to 7.05 % in H3, on average, resulting in a reduction of 20.1 %. Regarding TA, the mean values decreased from 6.30 g 100 mL^{-1} in H1 to 5.50 g 100 mL^{-1} in H3, showing a reduction of 12.7 %. These results followed those previously reported by Di Matteo et al. (2021) during the ripening of 'Femminello' lemons.

Table 1. Results of quality parameters analysis according to each harvest (H1, H2 or H3) and orchard (A or B).

Parameter	Samples	H1	H2	H3
TSS (%)	All	8.82 ± 0.89^{A}	7.90 ± 0.37^{B}	7.05 ± 0.73^{C}
	A	$9.59 \pm 0.56^{a*}$	$8.14 \pm 0.31^{b*}$	$7.48 \pm 1.01^{c*}$
	B	8.05 ± 0.26^{a}	7.66 ± 0.24^{b}	6.62 ± 0.67^{c}
TA (g 100 mL^{-1})	All	6.30 ± 0.99^{A}	5.78 ± 0.93^{B}	5.50 ± 0.76^{C}
	A	$6.94 \pm 1.00^{a*}$	$6.62 \pm 0.43^{b*}$	$6.18 \pm 0.28^{c*}$
	B	5.65 ± 0.36^{a}	4.94 ± 0.35^{b}	4.81 ± 0.38^{c}

Mean value ± standard deviation. Different capital letters in the same row mean significative differences between harvests ($p < 0.05$); different lowercase letters in the same row mean significative differences in each orchard between harvests ($p < 0.05$); * in each quality parameter at each harvest means significative differences between orchards ($p < 0.05$).

On average, in each harvest, lemons from A showed higher values of TSS (11.2 %) and TA (22.1 %) compared to lemons from B (Table 1). Hence, lemons harvested from

A were considered to have a better shelf life than those from B. As previously reported Sun et al. (2019), lemons with higher values of TSS and TA could be stored at 10 ºC for up to 90 days while still maintaining acceptable fruit quality compared to those with lower values.

3.2. Spectral features of intact lemons throughout ripening

Generally, the information provided by absorbance spectra regarding the internal quality of flesh fruit can be limited due to the depth of light penetration into the fruit skin (Rodríguez-Ortega et al., 2023). In the specific case of citrus fruit, light initially interacts with the flavedo and albedo layers before reaching the juice vesicles, complicating flesh quality prediction (Sun et al., 2020). In lemons, Ruggiero et al. (2022) observed that the NIR reflectance spectra of intact lemons predominantly reflected the spectroscopic characteristics of the flavedo, with minor influence from the albedo and even less from the endocarp tissues. Therefore, power intensity and the source-detector distance should be optimised to obtain maximal information about the layer of interest (Sun et al., 2020). Nevertheless, TSS and TA within the lemon juice follow the same evolution pattern as those determined in the peel (Serna-Escolano et al., 2022). Consequently, variations in absorbance across specific spectral regions of intact lemons during ripening could reveal the relationship between the external and internal attributes of the lemons.

During the ripening process, fruit experience changes in their chemical composition and structural properties, resulting in fluctuations in absorption levels throughout this process (Lu et al., 2020). Figure 1 shows the mean spectra of the lemon fruit from both orchards at each harvest. In both cases, the primary absorption wavelengths were observed at 970 nm, 1200 nm, and 1450 nm, as well as at 1350 nm, which overlapped at 1450 nm (Figure 1). As suggested by previous studies in citrus fruit (Liu et al., 2010;

Teerachaichayut and Ho, 2017; Ruggiero et al., 2022), the wavelengths around 970 nm and 1450 are attributed to OH bonds related to the water content, and the wavelengths around 1200 nm and 1350 nm are related to the CH, CH_2 and CH_3 groups present in polysaccharides, oils and waxes.

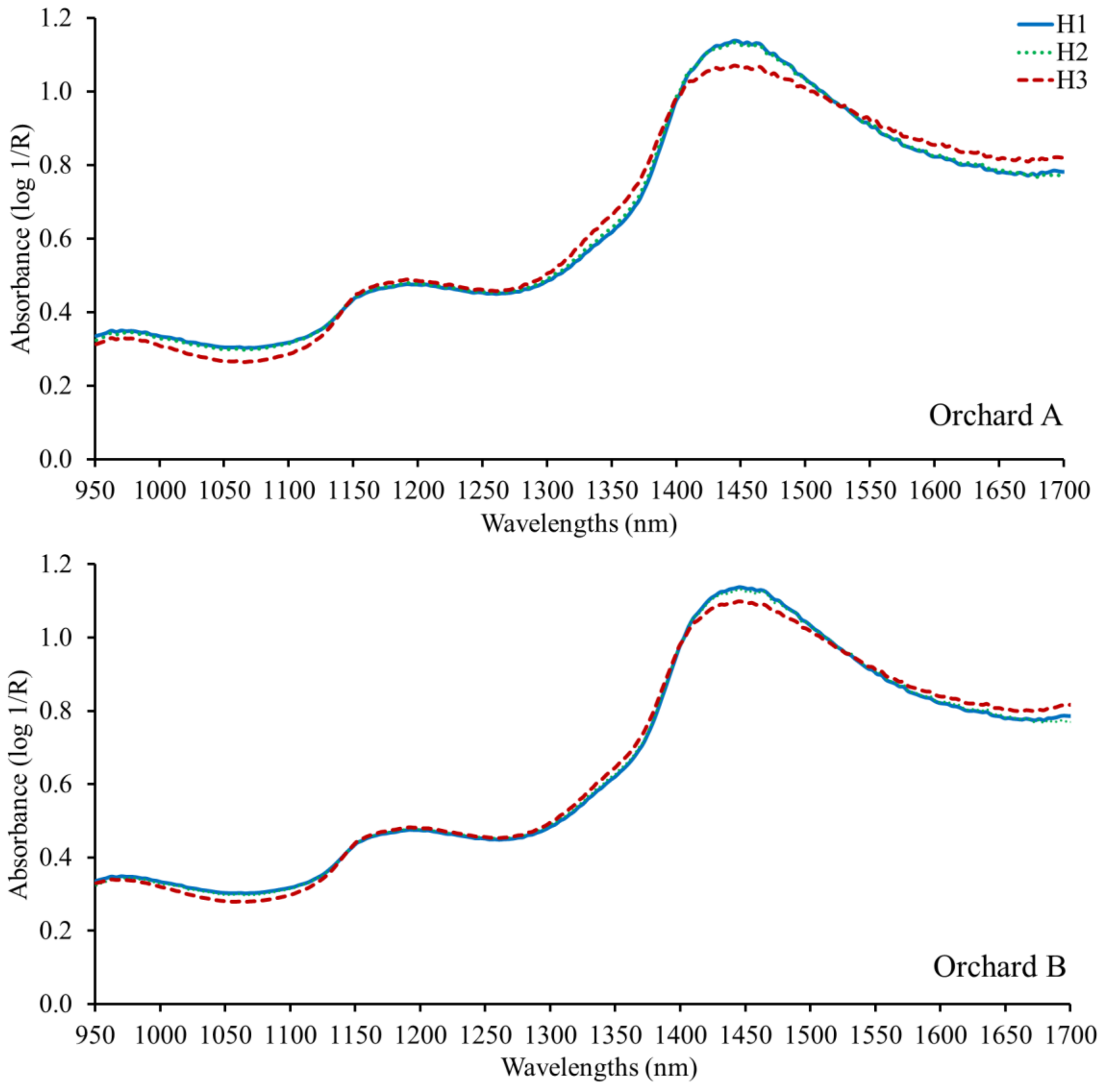


Figure 1. Mean absorbance spectra of intact lemons from each orchard according to the harvest timing.

Both orchards followed the same pattern of evolution, showing the major differences between the last harvest (H3) with respect to the first and the second harvest (H1-H2).

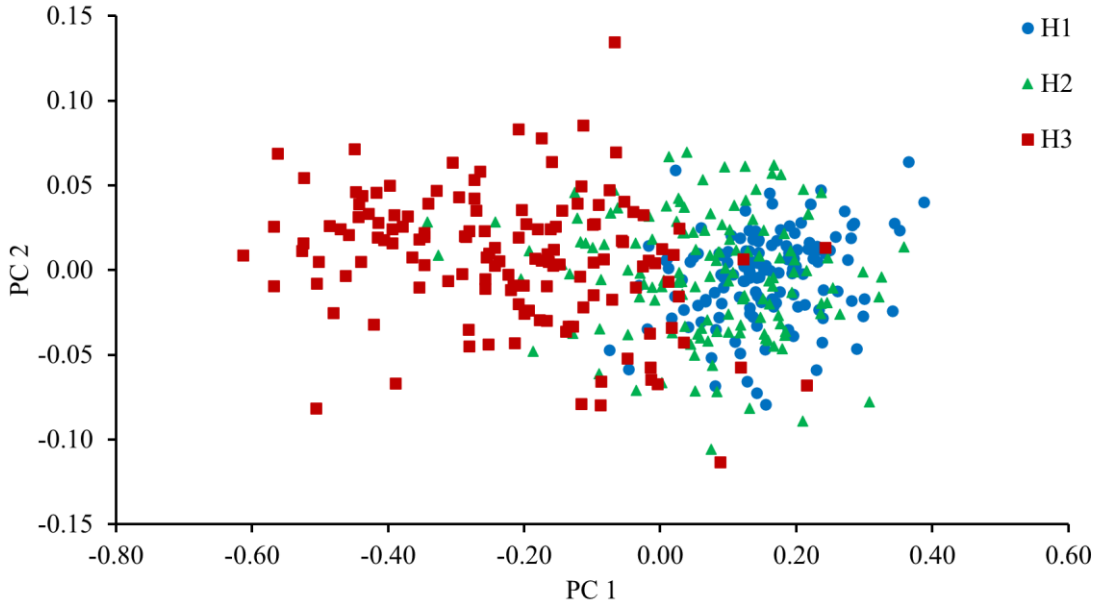
Regarding the orchard origin, lemons harvested from A showed greater differences compared to those from B. These differences were presented in the absorption valley of 1070 nm between the peaks of 970 nm and 1200 nm, where the absorbance decreased from harvests H1-H2 to H3; around peak 1350 nm, the absorbance increased from harvests H1-H2 to H3; at peak 1450 nm, where absorbance decreased from harvests H1-H2 to H3; and in the last absorption valley until the end of the studied range, where the absorbance increased from harvests H1-H2 to H3.

The flavedo of lemons is mainly composed of parenchymatous cells, cuticles, oil glands, and vascular bundles. This structure undergoes some changes during the maturation process on the tree. Some findings suggested that the wax of the cuticle changes its morphology during lemon maturation, influenced by environmental factors such as temperature and relative humidity (Romero & Lafuente, 2022). Also, the activity of phospholipases, which regulate membrane homeostasis through lipid turnover and membrane phospholipid degradation, increases in aged peels (Cronje et al., 2017). Thus, the lemon peel stability decreases during the maturation process and tissues reduce the capability control of the water balance, resulting in a superficial dehydration. Furthermore, previously published results showed that the firmness of on 'Fino' lemons decreased along the growth and developmental process (Serna-Escolano et al., 2022). In this sense, pectins, hemicellulose and cellulose are the primary cell wall components, and their depolymerisation by the main hydrolytic enzymes is directly related to fruit softening. This factor, bounded by the primary metabolism and the dehydration of the external fruit surface, allows some polysaccharides to increase in lemon flavedo during the ripening process (Serna-Escolano et al., 2021).

3.3. Exploratory analysis of the spectra of intact lemons

The analysis of the spectral data was performed by PCA using all samples from the two orchards and three harvests. The first two PCs of the PCA explained 95.8 % of the total variance (P1 = 87.8 % and PC2 = 8.0 %). To identify the various clusters in the distribution of the scores, they were coloured according to the harvest time and the orchard origin. Figure 2(a) shows the scores grouped by harvest time. H3 could be separated from H1 and H2 by PC 1. However, the samples of the H2 were overlapped between those of H1 and H3 and could not be separated using any of the first two PCs. According to the orchard aggrupation, Figure 2(b) shows that samples from both orchards overlapped and could not be separated for any PC. Therefore, the first two PCs showed differences between harvest time and ripeness but did not detect differences according to the lemon origin. This result could be explained since both orchards followed the same ripening pattern despite the absolute value of TSS and TA being higher in fruit from A, as Table 1 shows.

As PC1 from the PCA showed a separation between the less (H1) and more (H3) ripened (H1) samples, the loadings of this principal component, representing the influence of the wavelengths on this separation, were displayed in Figure 3. The wavelengths with the highest contributions were around 1450 nm, followed by 1070 nm and 1370 nm, and those at the end of the studied range. These wavelengths were the same or close to those that showed the greatest differences in the spectra of the fruits from both orchards related to the harvest timing (Figure 1), as previously analysed.



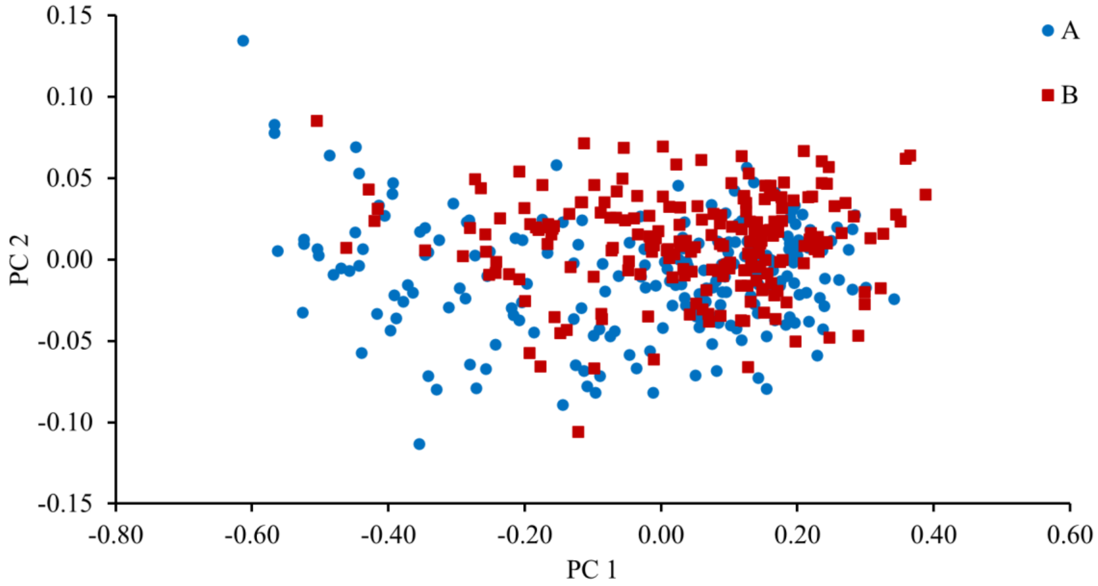


Figure 2. Score plots for the two first PCs of the PCA, coloured according to the harvest time (on the top) and the orchard (below).

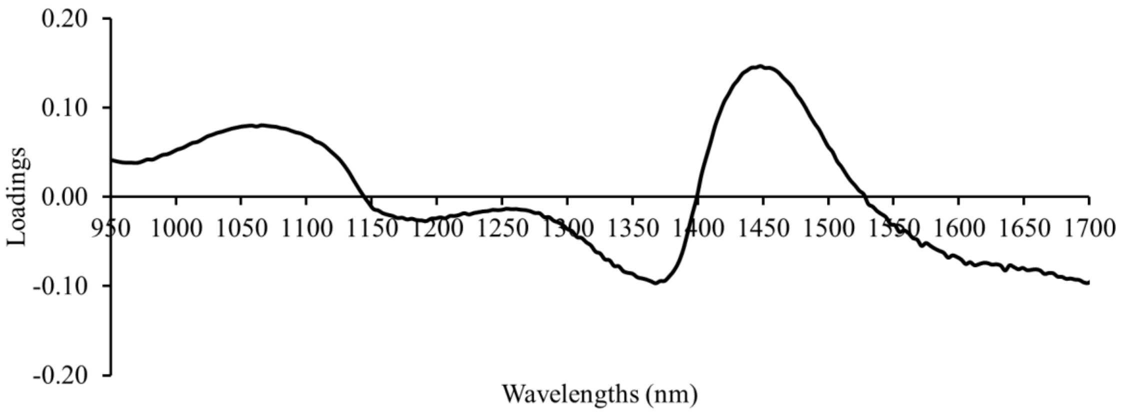


Figure 3. Loadings of the first PC of the PCA

3.4. Prediction of quality parameters

The content of TSS and TA was predicted through ripening using PLS-R calibrated models. The three approaches for validating the models were the internal, external, and external recalibrated models.

The internal validation approach presented the highest accuracies for both TSS and TA parameters and similar results in both calibration and validation sets (Table 3). The model was calibrated using 12 LVs for TSS, achieving an $R^2{}_P$ of 0.85 and $RMSE_P$ of 0.42 %. For TA, the model was also calibrated using 12 LVs, achieving an $R^2{}_P$ of 0.72 and an $RMSE_P$ of 0.45 g 100 mL^{-1}. In terms of RPD, the TSS model could be considered a good predictive model, while the TA model as suitable for approximate quantitative predictions.

When the external validation was performed, the models A/B and B/A resulted in a lower prediction ability for both parameters (Table 3), as previously reported in studies on mandarin (Pires et al. 2022) and oranges (Cavaco et al. 2018). In the case of TSS, the A/B and B/A models were calibrated using fewer LVs, 9 and 11, respectively. The accuracies in the prediction of the calibration set were similar to the internal approach or even better. The B-A model reached the lowest values of RMSEcv, 0.31 %. However, when both models were validated using the samples of the orchard not used for calibration, the accuracy decreased, resulting in values of R^2_P of 0.66 and 0.33 and values of $RMSE_P$ of 1.00 % and 1.13 %, for B/A and A/B model, respectively. For TA, both A/B and B/A models were calibrated also using fewer LVs, 6 and 9, respectively. The accuracy of A/B model in the calibration set was lower than the model using the internal approach, but the model B/A presented the lowest $RMSE_{CV}$ values in this set, 0.40 g 100 mL^{-1}. When the models were validated using the samples of the remaining orchard, the accuracy of both models was significantly reduced, resulting in values of R^2_P of 0.15 and 0.10 and values of $RMSE_P$ of 1.46 g 100 mL^{-1} and 1.47 g 100 mL^{-1} for B/A and A/B models, respectively. These models were considered unusable for the prediction of these parameters in lemons according to the RPD values, lower than 1.5.

Table 3. Results of prediction of TSS and TA content in the juice of the lemons following the three approaches for validation.

Parameter	App	Cal/Val	LVs	R^2_{CV}	$RMSE_{CV}$	R^2	$RMSE_P$	RPD
TSS (%) SEL = 0.1 %	1	A-B / A-B	12	0.84	0.41	0.84	0.42	2.5
	2	A / B	9	0.84	0.40	0.32	1.13	0.7
		B / A	11	0.81	0.31	0.66	1.00	0.7
	3	A_R / B	11	0.84	0.43	0.74	0.42	2.4
		B_R / A	12	0.84	0.33	0.67	0.58	1.7
TA (g 100 mL^{-1}) SEL = 0.04 g 100 mL^{-1}	1	A-B / A-B	12	0.72	0.43	0.72	0.45	2.0
	2	A / B	6	0.37	0.48	0.10	1.47	0.4
		B / A	9	0.54	0.40	0.15	1.46	0.3
	3	A_R / B	11	0.56	0.49	0.27	0.70	0.8
		B_R/ A	9	0.55	0.43	0.19	0.81	0.7

SEL = standard error of laboratory; App = approach (1 = internal validation; 2 = external validation; 3 = recalibration of the externally validated models); Cal = calibration; Val = validation; LVs = latent variables; RMSE= root mean square error; CV = cross-validation; P= prediction; RPD = ratio of performance to deviation; R = recalibrated; A = samples of orchard A; B = samples of orchard B

Therefore, the results of the present study indicate that prediction of TSS and TA of one orchard obtained by calibrating the models with only lemons from a different orchard was unsatisfactory. This is probably because when training with fruit from a single origin, the model only captures the particular characteristics of that origin and is not generally applicable. To train models that can be generalised, it is necessary to obtain fruit with different characteristics and origins. In this sense, a strategy to generalise the model was building the model using fruit from one origin and some samples from another (Pires et al. 2022). In the third approach, the models calibrated using the external validation were recalibrated using 10 % of samples from the orchard used to validate the models (Table 3). This approach greatly improved the results for the TSS model using external validation, especially for B/A model. In these recalibrations, similar accuracies to the external validation approach were obtained in calibrating the models. When the models were validated with the samples from the orchard not used for model calibration, values of $R^2{}_P$ of 0.74 and 0.67 and $RMSE_P$ of 0.42 % and 0.58 %, were obtained for A/B and B/A models, respectively. Using this approach, Pires et al. (2022) also improved the accuracy of the maturity index model, from RMSE values in the validation of 2.34 to 1.87, and Zude et al., (2008) in the TSS model, from 4.56 % to 0.86 %. When the TA models were recalibrated, the calibration accuracy of both models was similar to the external validation approach. In the validation, the accuracies improved as in TSS, particularly in the A/B model, achieving R^2_P and $RMSE_P$ values of 0.27 and 0.70 g 100 mL^{-1}. However, this improvement was not sufficient to achieve a precise model, only the

TSS models were considered suitable for approximate quantitatives predictions according to the RPD values.

The recalibration of the models using this strategy and their validation on the external dataset improved the results of TSS predictions, however the success for TA was still limited. This reduced accuracy may be attributed to the differences in the TA levels between the two orchards (Table 1). This fact was more evident when the correlation between measured and predicted TSS and TA parameters was plotted (Figure 4). Using internal validation resulted in calibration and validation set samples clustering along the trend line, as both sets used samples from both orchards and harvests. In the external validation using recalibrated models, for TSS, the samples from orchard B (validation set) were located at lower values but still within the range of samples from orchard A (calibration set). Instead, for TA, samples from the calibration (orchard A) and validation (orchard B) sets appeared totally separated, although some samples from the external set were used to recalibrate the model.

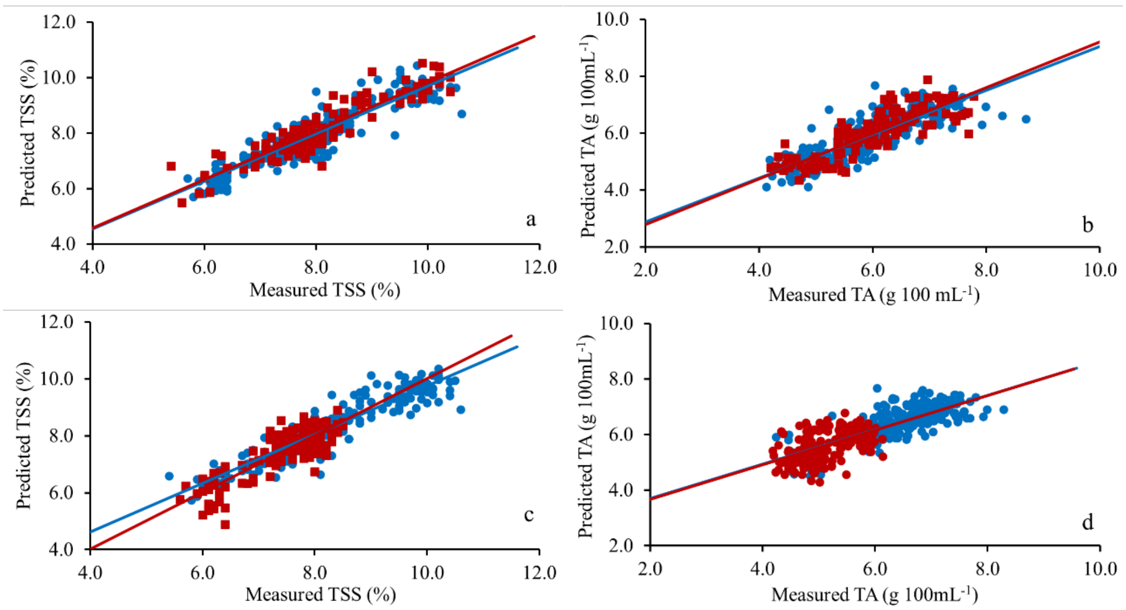


Figure 4. Predicted vs. measured values of TSS and TA. a: TSS (internal validation); b: TA (internal validation); c: TSS recalibrated model (calibrated using data from orchard

A and 10 % from B, and validated using data from orchard B); d: TA recalibrated model (calibrated using data from orchard A and 10 % from B, and validated using data from orchard B). Blue dots and trend line = calibration set; red dots and trend line = validation set.

It is not recommended to compare the results obtained in lemons with other studies on different citrus species due to physicochemical differences such as peel thickness, texture, and internal and external composition, which can influence spectroscopy measurements and consequently affect the results. (Cayuela et al., 2008; Jamshidi et al. 2012). In addition, in most cases, the studies carried out on citrus fruit presented results from models using only the standard internal validation without evaluating their robustness using an independent dataset. This is the case of the only study carried out on lemons (Ruggiero et al., 2022), in which only 119 samples obtained from only one harvest, from two consecutive seasons, two cultivars and seven locations were used for calibrating and validating the models. The spectral range 1000-2500 nm and multiple linear regression were used to build the models, obtaining values of $R^2{}_P$ of 0.16-0.90 for TSS and 0.37-0.95 for TA. Therefore, the results obtained in the current study following the three approaches were close to those of that study using only the internal validation. Nevertheless, several studies which also followed the external validation approach in other citrus species. In oranges, Cavaco et al. (2018) evaluated the quality of 'Newhall' variety fruit from two different orchards and seasons using a portable spectrometer in the range 680-1100 nm. In this study, internal and external validation were also carried out. In internal validation, when only one season was used, the values of RPD were 1.4 for both parameters, a value lower than in the current study. When external validation was performed, values of 1.1 and 0.9 were reached for TSS and TA, respectively. This difference concerning the current study was due to the high variability of the samples in the calibration models. In mandarins, Pires et al. (2022) studied the ripeness of 'Ortanique' variety using a portable system in the region 659–1136 nm. The fruit was collected from two different orchards at five different harvest times. Several models were developed using PLS-R and internal and external validations. During internal validation, TSS results were less precise than the current study and similar to TA, with RPD values of 1.8 and 1.9, respectively. External validation using different orchards resulted in a lower accuracy as in the current study, yet the accuracy was higher, with RPD values of 0.9 and 1.2 for TSS and TA, respectively. This can be attributed to the fact that the harvesting period of that study spanned six months, utilising fruit that could be either immature or overripe, leading to increased variability in the models of both quality parameters. In the present study, the fruit was harvested when they reached commercial maturity, at three ripeness stages over three months. Guthre et al., (2005) assessed the TSS of 'Imperial' variety using a spectrometer in the 720-950 nm spectral range. They employed different combinations of fruit collected on different days from different orchards and seasons to calibrate the models and validate them externally. When that study calibrated the models by location, the results achieved in the validation were more accurate using two locations but similar using only one.

3.5. Origin discrimination

Finally, PLS-DA models were employed to discriminate the origin of the orchard, and results are shown in Table 5. Similar to the quality parameters, both internal and external validations were carried out. All models effectively distinguished between orchards in the calibration set. Internal validation achieved a perfect discrimination rate of 100% upon validation. However, when the models were calibrated with samples from two harvests and validated with the samples from the remaining harvest, the accuracy decreased as in the prediction of quality parameters (Table 4). The most effective externally validated model achieved a correct classification rate of 66.4 %, in which H1 and H3 were used for calibration, representing less and more ripe fruit, respectively, while H2, or fruit with intermediate ripeness, was used for validation.

Table 5. Results of discrimination of lemon samples according to the orchard origin following first and second approaches.

Approach	Cal / Val	LVs	Orchard	Calibration (CV)			Validation		
				A	B	CC (%)	A	B	CC (%)
1	H1-H2-H3 / H1-H2-H3	13	A	140	0	99.3	70	0	100
			B	2	138		0	70	
2	H1-H2 / H3	11	A	140	0	100	27	43	57.1
			B	0	140		17	53	
	H1-H3 / H2	13	A	140	0	100	41	29	66.4
			B	0	140		18	52	
	H2-H3/ H1	12	A	140	0	100	40	30	57.1
			B	0	140		30	40	

Cal = calibration; Val = validation; LVs = latent variables; CC = correct classification; 1 = internal validation; 2 = external validation

A previous study distinguished full ripe 'Femminello' lemons with protected geographical indication (Ruggiero et al., 2022) using NIRS and models based on linear discriminant analysis. The results achieved discrimination rates similar to this study. However, they were obtained through only internal validations, but the dataset included fruit from several orchards and cultivars.

4. Conclusions

This work investigated the potential of NIRS in the 950-1700 nm range, combined with chemometrics, to determine the ripening and origin of 'Fino' lemons according to the TSS and TA levels. An exploratory analysis was conducted employing PCA, revealing that differences in the lemon spectra could be discerned based on their ripeness, not by their orchard origin. Using PLS-DA, 100 % and 67.4 % of the samples were correctly discriminated according to their origin using internal and external validation, respectively. The PLS-R models reached good results in predicting TSS and TA parameters using internal validation, achieving RPD values of 2.5 and 2.0, respectively. The accuracy decreased significantly when the models were validated with an independent set, especially in TA. However, when these models were recalibrated with several samples from the validation set, TSS could improve its accuracy, reaching an RPD value of 2.4.

Therefore, this study demonstrates that NIRS is a promising tool for lemon producers to manage the marketing properly, attending to the ripening stage of the fruits. Still, further research is required to enhance the robustness of calibration models to apply the technology at industrial level.

Authors contribution

Vicente Serna-Escolano: Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original draft preparation, Writing - Review & Editing**. María J. Giménez:** Investigation, Formal analysis. **Pedro J. Zapata:** Supervision, Writing - Review & Editing, Funding acquisition**. Sergio Cubero:** Resources, Formal analysis. **José Blasco:** Supervision, Writing - Review & Editing, Funding acquisition. **Sandra**

Munera: Methodology, Investigation, Formal analysis, Visualisation, Writing - Original draft preparation, Writing - Review & Editing.

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