

Document downloaded from:

<http://hdl.handle.net/10251/110348>

This paper must be cited as:

Cortes-Lopez, V.; Blasco Ivars, J.; Aleixos Borrás, MN.; Cubero García, S.; Talens Oliag, P. (2017). Visible and near-infrared diffuse reflectance spectroscopy for fast qualitative and quantitative assessment of nectarine quality. *Food and Bioprocess Technology*. 10(10):1755-1766. doi:10.1007/s11947-017-1943-y



The final publication is available at

<https://doi.org/10.1007/s11947-017-1943-y>

Copyright Springer-Verlag

Additional Information

Visible and near-infrared diffuse reflectance spectroscopy for fast qualitative and quantitative assessment of nectarine quality

Cortés, V.^{1,2}; Blasco, J. ²; Aleixos, N.³; Cubero, S.²; Talens, P.^{1*}

¹Departamento de Tecnología de Alimentos. Universitat Politècnica de València. Camino de Vera s/n, 46022, Valencia (Spain).

²Centro de Agroingeniería. Instituto Valenciano de Investigaciones Agrarias (IVIA). Ctra. Moncada-Náquera km. 4.5, 46113, Moncada, Valencia (Spain).

³Departamento de Ingeniería Gráfica. Universitat Politècnica de València. Camino de Vera s/n, 46022, Valencia (Spain).

*Corresponding author: Pau Talens. Departamento de Tecnología de Alimentos. Universitat Politècnica de València. Camino de Vera, s/n. 46022. Valencia. Spain. Phone: 34-963879836, e-mail: pautalens@tal.upv.es

ABSTRACT

Visible and near-infrared spectroscopy has been widely used as a non-invasive and rapid assessment technique for the quality control of agricultural products. In this study, 325 samples of nectarines representing two commercial varieties, cv. 'Big Top' and cv. 'Magique', were analysed by visible and near-infrared diffuse reflectance spectroscopy (VIS-NIR). The spectral data were pre-treated and analysed to predict the internal quality of the samples and to discriminate between the two varieties. Good prediction of the internal quality of the samples, using partial least squares regressions, was observed for both (R^2_P of 0.909 and 0.927 and RMSEP of 0.235 and 0.238 for cv. 'Big Top' and 'Magique', respectively). Discriminant models, using linear discriminant and partial least squares discriminant analyses were built to classify the nectarines. Both methods provided good results with rates of 97.44 % and 100 % of correctly classified samples. The results indicated that visible and near infrared techniques can be useful and simple methods for quality control and for the correct identification of nectarines in commercial lines as an alternative to the slower and less accurate manual classification.

Keywords: fruit quality; spectroscopy; nectarine; chemometrics; prediction; discrimination

1. INTRODUCTION

Nectarine (*Prunus persica* var. *nucipersica*), is one of the most dynamic species of fruit in terms of the emergence of new varieties on the market. This dynamism has contributed to the development of improved varieties that are better adapted to the growing conditions and market requirements. In terms of evolution and according to the type of fruit, nectarine is the most important fruit group with 41 % of total production, followed by peaches (35 %) and clingstone peaches (24 %) (Iglesias, 2013). The total estimated production of European nectarine was over 1,508,288 tonnes in 2012, shared among Italy (53 %), Spain (32 %), France (10 %), and Greece (5 %) (GenCat, 2013). This high productivity has triggered the necessity to verify nectarine varieties in the industrial fruit packing-line. For example, in Spain, in the region of Lérida the optimal harvesting time is in the summer season (between 1st June and 30th September). At the end of July more than 20 nectarine varieties are being picked simultaneously across the entire region. In the specific case of the village of Aitona (south of Lérida), in practice, the largest number of nectarine varieties that may be harvested simultaneously is six, all of which could reach the post-harvesting industry concurrently in average amounts of 500 tonnes/day, thus generating huge problems in the classification of varieties. In addition, it must be taken into account that nowadays, growers can choose between a large range of nectarine varieties adapted to the climate, different harvest periods and the agronomic characteristics of each particular area. The combination of a large number of small orchards and a wide range of varieties with different demand (Bonany *et al.*, 2013) and market value, can become a source of potential problems for the local post-harvesting industry because of the involuntary or fraudulent mixing of different fruit varieties (Font *et al.*, 2014).

Additionally, the commercial-scale introduction of yellow flesh colour varieties with a strong colour and sweet flavour, as is the variety 'Big Top', represented a remarkable innovation for its sweet taste (<6 gL⁻¹ of malic acid) and the excellent consistency of the fruit, being widely accepted by consumers. Satisfying consumers demands a key aspect, which is the selection of good indicators of quality. Previous works have shown the relationship between consumer acceptance and a high concentration of total soluble solids (TSS) or other factors like the acidity, TSS/acidity relationship or phenolics and volatile substances (Crisosto *et al.*, 1997; Crisosto *et al.*, 2002; Crisosto *et al.*, 2003).

Nectarine is a climacteric fruit and therefore the physicochemical changes produced during postharvest will determine the final status of the product quality. Only the accurate control of these changes can ensure the customer enjoys good organoleptic and sensorial quality. Traditionally, the internal quality monitoring of the

fruit has been performed using destructive methods, so that only a small number of pieces can be measured per set, but the observations may differ greatly from the real status of the whole of production (Valero *et al.*, 2007). One of the aims of the postharvest sensing technologies, such as computer vision (Cubero *et al.*, 2011) hyperspectral imaging (Lorente *et al.*, 2012) or near-infrared (NIR) spectroscopy (Nicolai *et al.*, 2007), is to allow the analysis of the whole production in terms of quality and commercial organoleptic, nutritional and health characteristics, and varietal verification, while losses and process cost are minimised (Ferrer *et al.*, 2001). The use of sensors based on NIR technology, along with chemometric data models, is one of the fastest and cleanest techniques to achieve this aim. The literature contains different studies on the applicability of NIR technology to the analysis and classification of nectarine varieties (Carlomagno *et al.*, 2004; Pérez-Marín *et al.*, 2011; Reita *et al.*, 2008). For example, Pérez-Marín *et al.* (2011) evaluated the ability of different NIR instruments, to classify intact nectarines cv. 'Sweet Lady' according to internal quality in postharvest storage as a function of pre-harvest irrigation strategies. Sánchez *et al.* (2011) predicted of some quality parameters (weight, diameter, soluble solid content and flesh firmness), both on-side and in-line, in nectarines cv. 'Sweet Lady' with different harvests and crop practices. Reita *et al.* (2008) developed different methods for °Brix determination of nectarine cv. 'Big Bang Maillar', cv. 'Sweet Red' and cv. 'Nectaross'. Peiris *et al.* (1998), Golic & Walsh (2006) and Ma *et al.* (2007), determined the sugar content of peaches, while Fang *et al.* (2013) determined sugar content, acidity and water content in yellow peach between 350 - 2500 nm and achieved $R^2 > 0.61$ for all properties except acidity determination, which still needs to be improved. These previous works have conducted studies on the use of VIS-NIR technology to assess the internal quality of stone fruits but they focus only on certain properties. The use of quality indexes can correlate these properties. For instance, the IQI index (Cortés *et al.*, 2016) includes different analytical parameters, specifically three parameters typically employed in postharvest handling to evaluate the quality of this fruit. The study by Carlomagno *et al.* (2004) already assessed ripeness of peaches according to the combination of firmness and sugar content. The IQI, however, has the advantage of including the visual component (internal colour), which is a property of great importance to the consumer, in addition to the compositional aspects (firmness and soluble solids content), but avoiding the titratable acidity analysis because it is a laborious and slow process that generates waste. Indeed, one of the main benefits of the IQI is that all the required analyses are less time-consuming, with less pre-treatment of the sample and lower costs, and can be assessed using VIS-NIR diffuse reflectance spectroscopy.

This study aims to evaluate the performance of VIS-NIR reflectance spectroscopy as a tool to predict the internal quality of nectarines, and the potential of the information obtained to differentiate among varieties with different

commercial interest. To this end, two varieties with a similar composition, grown in the same period, but with different development, cv. 'Big Top' and cv. 'Magique', have been analysed.

2. MATERIALS AND METHODS

2.1. Experimental procedure

The experimental part of this paper was carried out using 325 fruits of two commercial varieties of nectarines, cv. 'Big Top' and cv. 'Magique', harvested in a commercial orchard in Lérida, Spain. These varieties were chosen because they represent 36 % of the overall Spanish nectarine production (Font *et al.*, 2014), are grown at the same time and have a similar composition and organoleptic properties. Both are classified as a melting (slow-melting) phenotype, but present a large degree of variability in terms of development and maturation speed, and must therefore be handled differently in postharvest.

The samples were free from visual damage and with had a uniform size and colour. On arrival at the laboratory, fruits were cleaned, individually numbered and randomly divided into sets of 25 fruits each. All sets were stored at 15 °C to simulate the room conditions allowing the gradual maturation of the product. As the 'Magique' variety ripens more slowly than 'Big Top', a total of six sets were analysed for the 'Big Top' variety on the 1st, 2nd, 3rd, 4th, 5th and 9th days, while a total of seven sets were analysed for the 'Magique' variety on the 1st, 3rd, 5th, 8th, 11th, 15th and 17th days. The VIS and NIR spectra of the fruits in each set were collected and their physicochemical properties were analysed by standard destructive methods.

2.2. Visible and near-infrared spectra acquisition

The visible spectra of the fruits were collected using a conventional spectrophotometer (CM-700d, Minolta Co., Tokyo, Japan) every 10 nm between 360 nm and 700 nm. The VNIR and NIR spectra were collected using a multichannel VIS-NIR spectrometer platform (AvaSpecAS-5216 USB2-DT, Avantes BV, The Netherlands) equipped with two detectors (Fig. 1), one sensitive in the range from 595 nm to 1100 nm with a spectral FWHM (full width at half maximum) resolution of 1.15 nm and a spectral sampling interval of 0.255 nm (AvaSpec-ULS2048 StarLine, Avantes BV, The Netherlands) and the other sensitive in the NIR range from 888 nm to 1795 nm with a spectral FWHM resolution of 12 nm and a spectral sampling interval of 3.535 nm (AvaSpec-NIR256-1.7 NIRLine, Avantes BV, The Netherlands). The measurements were performed using a bi-directional fibre-optic reflectance probe (FCR-7IR200-2-45-ME, Avantes BV, The Netherlands). The probe is configured with an illumination leg with six 200 µm fibre cables which connects to a fibre-coupled light source and a single

200 µm read fibre cable to measure the diffuse reflectance via connection to a spectrometer. A 10 W tungsten halogen light source (AvaLight-HAL-S, Avantes BV, The Netherlands) was used to ensure a constant light intensity over the whole measurement range. The probe tip is designed to enable diffuse reflectance measurements under an angle of 45 ° to prevent direct back reflection from the surface of the fruit. A personal computer equipped with commercial software (AvaSoft version 7.2, Avantes, Inc.) was used to control both the detectors and to acquire the spectra. The integration times were adjusted for each spectrophotometer using a 99 % reflective white reference (WS-2, Avantes BV, The Netherlands), so that the maximum reflectance value over each wavelength range was around 90 % of saturation (Lorente *et al.*, 2015). They were set to 120 ms for the first detector and 500 ms for the second one. To reduce the thermal noise of the detector, each spectrum was obtained as the average of five scans (Nicolai *et al.*, 2007). The average reflectance measurements of each sample (S) were then converted into relative reflectance values (R) with respect to the white reference using dark reflectance values (D) and the reflectance values of the white reference (W), as shown in Equation (1):

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

The dark spectrum was obtained by turning off the light source and covering the tip of the reflectance probe.

Prior to spectral measurements, the temperature of the nectarines was stabilised at a room temperature of 22 ± 1 °C. All the measurements were performed by placing the skin of the fruit on the equipment. Measurements were taken at two points on each side of fruit and mean values of the spectra were used for the analysis.

2.3. Determination of quality attributes

Standard destructive quality testing methods were performed immediately after the acquisition of the spectral measurements to determine quality attributes for use as reference values. Flesh colour was determined with the spectrophotometer using the standard illuminant D65 and the 10° observer for all colour measurements. Colour attributes such as luminosity (L*), chromaticity (C*) and hue angle (h*) were obtained from the CIELAB colour space. L* was obtained directly by the spectrophotometer whereas C* and h* were estimated by Equations 2 and 3, respectively.

$$h^* = \arctg \frac{b^*}{a^*} \quad (2)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

a* and b* being the CIELAB attributes.

Firmness of the nectarines was measured using a Universal Testing Machine (XT2 Texture Analyser, Stable Microsystems, Haslemere, England) to perform puncture tests using a 6 mm diameter cylindrical probe

(P/15ANAMEsignature) to a relative deformation of 30 % at a speed of 1 mm/s. Two measurements were performed for each fruit on opposite sides along the equator. The fracture strength (F_{max}) was analysed for all samples, expressed the maximum force, in Newtons, applied to break the sample.

Immediately after firmness measurements, samples of nectarine juice were extracted to estimate the TSS by refractometry (°Brix) with a digital refractometer (RFM330+ set, VWR International Eurolab S.L. Barcelona, Spain) at 20 °C with a sensitivity of ± 0.1 °Brix. Samples were analysed in triplicate and average values were calculated.

Subsequently, the multi-parameter internal quality index (IQI, Cortés *et al.* (2016) was calculated by Equation 4.

$$IQI = \frac{\ln(100 \cdot F_{max} \cdot L^* \cdot h^*)}{TSS \cdot C^*} \quad (4)$$

where F_{max} is the fracture strength (Newton), TSS is the total soluble solids (°Brix) and L^* , h^* , C^* are the colour attributes of the colour of the flesh.

2.4. Spectral pre-processing

The raw spectra from each of the three measuring devices were normalised (Bakeev, 2005) by dividing each variable by its standard deviation. In this way, the spectral intensities are rescaled to a common range, thus making it possible to compare the spectra acquired using different pieces of equipment with different resolutions. Then, the spectra were transformed to apparent absorbance ($\log(1/R)$) values to linearise the correlation with the concentration of the constituents (Hernández *et al.*, 2006) using The Unscrambler V10.3 software package (CAMO, Norway). In addition, two pre-processing techniques were applied: Savitzky-Golay smoothing with a gap of three data points (Carr *et al.*, 2005) combined with extended multiplicative scatter correction (EMSC) (Martens *et al.*, 2003; Bruun *et al.*, 2007). Smoothing, which includes moving smoothing and Savitzky-Golay smoothing, is one of the methods that are most often used to eliminate noise (Gorry, 1990; Savitzky & Golay, 1964), and EMSC is a method that is well suited to the removal of physical effects from chemical information, i.e. it is particularly useful for minimising wavelength-dependent light scattering variation (Santos *et al.*, 2013). Figure 2 shows raw VIS-NIR spectra and their correction after the application of the pre-processing methods.

2.5. Chemometric data treatment

A one-way analysis of variance (ANOVA) was conducted to determine significant differences in the physicochemical properties (F_{max} , TSS, L^* , h^* , C^* and IQI) during the postharvest evolution of the fruit using the software Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD, USA). The multivariate

analysis was performed through partial least squares discriminant analysis (PLS-DA) and linear discriminant analyses (LDA) were performed using the Unscrambler X software package.

2.5.1 Prediction analysis of internal quality

Two matrices were created for each variety of nectarines, where the rows represented the samples (#N = 150 for cv. 'Big Top' and #N = 175 for cv. 'Magique') and the columns represented the variables (X-variables and Y-variables). The X-variables, or predictors, were the different VIS-NIR spectra while the Y-variable, or response, was the IQI estimated for each sample. Before calibration, principal component analysis (PCA) was performed to extract the most important information about spectral data and to exclude samples considered outliers.

Two regression models for each variety of nectarines were developed by partial least squares (PLS) to predict the IQI based on the spectral measurements. This method is often used in spectroscopy analysis to evaluate the quality characteristics of intact fruits, for example, mandarin (Hernández *et al.*, 2006), tomato (Shao *et al.*, 2007), orange (Cayuela & Weiland, 2010), mulberry (Huang *et al.*, 2011) and banana (Jaiswal *et al.*, 2012). Samples were randomly separated into two groups: 75 % of the samples were used for development and evaluation by a cross-validation model using the leave-one-out cross technique (Huang *et al.*, 2008), while the remaining samples (25 %) were used as the prediction set (Kamruzzaman *et al.*, 2012). The root mean square error of calibration (RMSEC), root mean squared error of cross validation (RMSECV), root mean square error of prediction (RMSEP), coefficient of determination for calibration (R^2_C), coefficient of determination for cross-validation (R^2_{CV}) and for prediction (R^2_P), and the required number of latent variables (LV) were used to judge the accuracy of the PLS model.

2.5.2. Discriminant analysis for varietal differentiation

Discriminant models, using LDA and PLS-DA were built to classify nectarines in terms of variety. A training set consisting of a random selection of 75 % of the studied samples was used to develop a qualitative calibration model. Each model was validated using the leave-one-out cross-validation technique (Huang *et al.*, 2008) and the weight of the spectral variables selected was $1/Sdev$. A test set (25 % of remaining samples) was used for the evaluation and comparison of the classification models (Soares *et al.*, 2013). These discriminant analyses seek to correlate spectral variations (X) with defined classes (Y), with attempts being made to maximise the covariance between the two types of variables. In this type of approach, the Y variables used are not continuous, as they are in quantitative analysis, but rather categorical "dummy" variables created by assigning different values to the

different classes to be discriminated. In the case of PLS-DA, the Y-variable was a vector with zeroes (for the cv. 'Big Top') and ones (for the cv. 'Magique'). However, for LDA the number of samples in the training set must be larger than the number of variables included in the model (Kozak & Scaman, 2008; Sádecká *et al.*, 2016), thus requiring a variable reduction. This was performed using the PCA scores as input data, since linear combinations of the original variables called principal components (PCs) are uncorrelated (Rodríguez-Campos *et al.*, 2011). In this study, the first seven principal components were used to replace the original one data (He *et al.*, 2006). The RMSEC, RMSECV, R^2_C , R^2_{CV} , LV and percentage of correctly classified samples were used to evaluate the discriminating capacity of the models.

3. RESULTS AND DISCUSSION

3.1. Analysis of the quality attributes

The changes observed in the firmness and TSS of the two varieties of nectarines during postharvest storage are shown in Figure 3.

As expected, the changes in both parameters during ripening are indicative of the physiological development of the fruit (Valero *et al.*, 2007). In both cultivars a steady decrease was observed in fruit firmness over time from around 47 N to 9 N for cv. 'Magique', and from 58 N to 6 N for cv. 'Big Top'. These values coincide with those reported by Ghiani *et al.* (2011) for nectarine cv. 'Big Top'. The textural changes that took place in the fruit during the postharvest period can be attributed to different factors, such as significant changes in the composition and structure of cell walls and, particularly, the degradation of the polysaccharides. As a result, the decrease in firmness during the process is due to a loss of neutral sugars, solubilisation and de-polymerisation of the polysaccharides of the cell wall, and the reorganisation of their interconnections (Singh *et al.*, 2013). The firmness values cover all the commercial ranges proposed by Crisosto (2002) and Valero *et al.* (2007), which are less than 18 N (ready to eat), between 18 and 35 N (ready to buy) and over 35 N (immature).

On the other hand, TSS increased continuously, from 10 ± 1 to 15 ± 3 during postharvest storage for cv. 'Big Top', which is nowadays the reference cultivar, known for quickly reaching its typical mature colour, sweet taste and optimum fruit size (Iglesias & Echeverría, 2009). This increment is due to the conversion of starch to glucose and fructose, which are used as substrates during fruit respiration (Eskin *et al.*, 2013). However, the increase in TSS observed for cv. 'Magique' remained around 10-11 % probably because this variety has a different pattern of ripening. However, in all cases, the values were greater than 8 °Brix, which is the minimum established by the European Union to market peaches and nectarines (R-CE No. 1861/2004). Several authors

have reported a linear relationship between TSS and consumer acceptance (Crisosto & Crisosto, 2005), a TSS of below 10 % generally being unacceptable to consumers (Clareton, 2000).

Figure 4 shows the evolution of the flesh colour of the two varieties of nectarines during the postharvest storage. Flesh colour was taken as the evaluation parameter rather than external colour because for some nectarine varieties with early development of the external colour, such as cv. 'Big Top', their external colour should not be used as a maturity index (Ravaglia *et al.*, 1996) because they reach the appearance of being mature before they are ready to eat (Della Cara, 2005; Iglesias & Echeverría, 2009). In this sense, other authors such as Tijssens *et al.* (2007) suggest that flesh colour is a good index to determine the development of the fruit. It can be observed that L^* and h^* decrease and C^* increases during the storage for both varieties. Cv. 'Big Top' changes from $L^* = 72$, $h^* = 79$ and $C^* = 42$ in the unripe stage until $L^* = 62$, $h^* = 74$ and $C^* = 60$ at the states of further development, and cv. 'Magique' changes from $L^* = 72$, $h^* = 102$ and $C^* = 24$ to $L^* = 70$, $h^* = 86$ and $C^* = 29$. Figure 4b shows an example of the internal appearance of both cultivars on each day of analysis. The flesh colour changed from whitish-yellow to orange-red for the cv. 'Big Top' and from whitish-green to yellow-orange during postharvest storage especially for the cv. 'Magique', similar to the findings of Padilla-Zakour (2009), who reported that the colour of peaches changed from yellow-greenish to yellow-orange or orange-reddish when fruits matured.

Figure 5 shows the evolution of the IQI, which is represented by a sigmoidal curve for both varieties. The values of the indices clearly decreased during the storage period for cv. 'Big Top' (Fig. 5a) and for cv. 'Magique' (Fig. 5b), but three trends can be differentiated in the graph. Initially, the two IQI decline slowly until 17.5 because the maturation of the product has not yet been produced at the beginning of the IQI curve, and then drops sharply when the fruits ripen to achieve their optimum organoleptic properties, which are related to adequate firmness, content in TSS and flesh colour. Finally, fruit reach the stage of over-ripeness, where the curve follows a constant trend because the product reaches a maximum TSS content and a minimum firmness. It should be noted that, even though they have the same trends, the two varieties have different maturation speeds. Thus, while cv. 'Big Top' nectarines reach over-ripeness at day 5 (E in Fig. 5a), cv. 'Magique' nectarines do so at day 15 (F in Fig. 5b).

3.2. Spectral analysis

Figure 6 presents the VIS-NIR absorbance spectra of each day of storage for both varieties. Each spectrum represents an average of the measurements done. As can be seen, there was considerable spectral similarity between the varieties analysed, due to the existing features being very similar in their chemical structures. Even, the pattern of the absorption curves is similar to other fruits such as pear (Liu *et al.*, 2008), açai and juçara fruits (Cunha *et al.*, 2016), peach (Martins *et al.*, 2016) and mandarin (Magwaza *et al.*, 2012), although the position and magnitude of the peaks are specific for each fruit.

From the visible region (360 – 770 nm), a continuous decrease in absorbance, with the minimum at 680 nm, is observed. The spectra show a broad absorbance band around 450 nm associated with carotenes and xanthophylls (Lichtenthaler & Buschamann, 2001). The high absorbance observed around 670 nm is indicative of the presence of chlorophyll, which gives the fruit its characteristic green colour (Merzlyak *et al.*, 2003; Gómez *et al.*, 2006). Furthermore, Tijssens *et al.* (2007) concluded that absorption at 670 nm allowed the evaluation of the variation in maturity of individual nectarines. The peaks centred at 970 nm and 1400 nm that appear are probably due to the presence of water (Williams & Norris, 1987; McGlone & Kawano, 1998). A characteristic absorption band at around 1160 nm was sugar-related (Osborne *et al.*, 1993; Walsh *et al.*, 2004). Lu (2004) stated that the absorption of radiation increases as fruit firmness decreases, i.e. firmer fruit reflect more radiation than softer fruit. Fu *et al.* (2007) stated that this is also linked to water and pectin content.

3.3. Analysis of internal quality

Table 1 displays the results for the calibration and prediction models of the internal quality of intact nectarines.

PLS models built for both varieties showed similar prediction coefficients and performance. The best method is usually the one that minimises the prediction error (RMSEP) and number of LV for an independent test set (Faber, 1999) while maximising the R^2_p . In this case, when applied to an independent prediction set, the PLS models were capable of predicting IQI with R^2_p of 0.909 and 0.927 and RMSEP of 0.235 and 0.238 for cv. ‘Big Top’ and ‘Magique’, respectively. Figure 7 shows a good prediction performance of the PLS models for IQI. These results suggest that the calibration models optimised with leave-one-out cross-validation are representative and the models can accurately predict unknown sample data.

These results are similar to those obtained by Cortés *et al.* (2016), who have recently developed models to predict the internal quality of mangoes cv. ‘Osteen’ using the IQI ($R^2_p = 0.833$). Thus, it is confirmed that the IQI can be applied to various types of fruits to ensure an adequate quality of the final product for the consumer.

3.4. Varietal classification

Before performing discriminant analysis, a PCA decomposition was conducted to recognise any possible pattern of classification. The spectrum of each sample was represented as a point, with respect to these new axes (Downey, 1997). The samples of cv. 'Magique' were used to develop the PCA model used later and to project the samples of cv. 'Big Top'. Figure 8 shows the two-dimensional scatter plot of scores for two principal components (PCs) from projection results. The two PCs explain over 70 % (53 % for the first PC and 18 % for the second PC) of the variation. This justifies the possibility of differentiating between varieties using the spectra measured in intact nectarines.

After proving the performance of an unsupervised method such as PCA to classify samples belonging to different varieties, the next step consisted in building classification models based on supervised LDA and PLS-DA. The best models were chosen with seven LV for both cases. The optimal number of latent variables was chosen according to the lowest RMSECV by internal validation, i.e. 'leave-one-out', in combined analysis with the cumulative variance in the X and Y blocks (Bachion de Santana *et al.*, 2016).

All the training set and validation samples were correctly classified by the PLS-DA model, as shown in Figure 9. In this situation, all cv. 'Big Top' samples have predictive values close to, 1 thus classifying these as belonging to class '1', and cv. 'Magique' samples have predictive values close to 0, therefore classifying these as belonging to class '0'. The values of the RMSEC and RMSEP were 0.112 and 0.133, respectively, which exhibit a good agreement, indicating that the calibration error is a good estimation of the standard error of prediction observed in samples in the test set. Moreover, the validation set gave a similar result to the calibration set, with R^2 of 0.949 and 0.926 respectively, thereby indicating good performance of the model for varietal classification.

Regarding LDA, Figure 10 shows the results of the external validation by test set (25 %) of each variety. Validation samples of the cv. 'Big Top' are displayed in blue, while samples of the cv. 'Magique' are in red. The classification accuracy was 97.44 %, all cv. 'Magique' samples being classified correctly and only two cv. 'Big Top' samples misclassified.

Table 2 shows the summary of the classification accuracy for each analysis, presented as both as percentage and absolute number of correctly classified samples.

As both varieties have a similar composition, to develop a tool capable of differentiating them is challenging and could be possible to be applied to differentiate other varieties with greater difference. Based on these results, the ability of these VIS-NIR instruments to classify fruit as depending on its variety for compositionally similar

samples has been demonstrated satisfactorily for nectarine. So, it is possible to explain the postharvest shelf-storage time using these techniques for varieties with different speeds of evolution because they are classified correctly from their origin.

4. CONCLUSIONS

The quantitative and qualitative results of this study confirmed that VIS-NIR spectroscopy is a technique capable of determining the internal quality of intact nectarines with significant reliability. The partial least squares regression analysis showed strong performance in predicting the internal quality of the samples, with an R^2_P and RMSEP of 0.909 and 0.235 for cv. 'Big Top', and 0.927 and 0.238 for 'Magique'. It has been possible to differentiate three trends of the IQI curve, where initially the maturation of the product has not yet been produced, followed by the development of the optimum organoleptic properties, and finally the fruit reaches the stage of over-ripeness. Despite being two varieties with a similar composition and grown in the same period, it was possible to separate the two with a perfect classification rate of 100 % using PLS-DA and 97.44 % using the model developed by LDA models. This represents an advance in the creation of tools for monitoring the fruit quality for the postharvest industry compared to the present situation where the evaluation of the state of the fruit is mostly carried out based on the subjective experience of trained experts. Further studies are needed to improve the calibration specificity, accuracy and robustness, and to extend the discrimination to other nectarine varieties.

5. Acknowledgements

This work was partially funded by the Generalitat Valenciana through project AICO/ 2015/122 and by INIA and FEDER funds through projects RTA2012-00062-C04-01 and 03, and RTA2015-00078-00-00. Victoria López Cortés thanks the Spanish Ministry of Education, Culture and Sports for the FPU grant (FPU13/04202). The authors are also grateful to Fruits de Ponent (Lérida) for providing the fruit.

6. REFERENCES

- Bachion de Santana, F., Caixeta Gontijo, L., Mitsutake, H., Júnior Mazivilla, S., Maria de Souza, L. & Borges Neto, W. (2016). Non-destructive fraud detection in rosehip oil by MIR spectroscopy and chemometrics. *Food Chemistry* 209, 228-233.
- Bakeev, K. A. (2010). *Process Analytical Technology*. United Kingdom: Wiley.

- Bonany, J., Buehler, A., Carbó, J., Codarin, S., Donati, F., Echeverria, G., Egger, S., Guerra, W., Hilaire, C., Höller, I., Iglesias, I., Jesionkowska, K., Konopacka, D., Kruczynska, D., Martinelli, A., Pitiot, C., Sansavini, S., Stehr, R., Schoorl, F., 2013. Consumer eating quality acceptance of new apple varieties in different European countries. *Food Qual. Prefer.* 30, 250–259.
- Bruun, S.W., Sondergaard, I. & Jacobsen, S. (2007). Analysis of protein structures and interactions in complex food by Near-Infrared spectroscopy. 1. Gluten Powder. *J. Agric. Food Chem.* 55,7234-7243.
- Carlomagno, G., Capozzo, L., Attolico, G. & Distante, A. (2004). Non-destructive grading of peaches by near-infrared spectrometry. *Infrared Physics & Technology* 46, 23-29.
- Carr, G.L., Chubar, O. & Dumas, P. (2005). *Spectrochemical Analysis Using Infrared Multichannel Detectors* 1st edn. (eds., Bhargava, R. & Levin, I.W.) 56–84. Oxford: Wiley-Blackwell.
- Cayuela, J. A. & Weiland, C. (2010). Intact orange quality prediction with two portable NIR spectrometers. *Postharvest Biology and Technology* 58 (2), 113-120.
- Clareton, M. (2000). Peach and nectarine production in France: trends, consumption and perspectives. *Summaries Prunus breeders meeting, EMBRAPA, clima temperado Pelotas (RS)*, 83–91.
- Commission Regulation EC, No.1861/2004 of 28 October 2004.
- Cortés, V., Ortiz, C., Aleixos, N., Blasco, J., Cubero, S., & Talens, P. (2016). A new internal quality index for mango and its prediction by external visible and near infrared reflection spectroscopy. *Postharvest Biology and Technology*, 118, 148–158.
- Crisosto, C. & Crisosto, G. (2005). Relationship between ripe soluble solids concentration (RSSC) and consumer acceptance of high and low acid meeting flesh peach and nectarine (*Prunus persica* (L.) Batsch) cultivars. *Postharvest Biol. Technol.* 38, 239–246.
- Crisosto, C. H., Crisosto, G. M. & Metheney, P. (2003). Consumer acceptance of ‘Brooks’ and ‘Bing’ cherries is mainly dependent on fruit SSC and visual skin color. *Postharvest Biol. Technol.*, 28, 159–167.
- Crisosto, C. H., Crisosto, G. M., Ritenour, M. A. (2002). Testing the reliability of skin color as an indicator of quality for early season ‘Brooks’ (*Prunus avium*L.) cherry. *Postharvest Biol. Technol.*, 24, 147–154.
- Crisosto, C. H., Garner, D., Crisosto, G. M., Wiley, P., & Southwick, S. (1997). Evaluation of the minimum maturity index for new cherry cultivars growing in the San Joaquin Valley. Visalia, CA: California Cherry Growers Association.
- Crisosto, C.H. (2002). How do we increase peach consumption? *Proceedings of 5th International Symposium on Peach, ISHS, Acta Hort.* 592, 601–605.

- Cubero, S., Aleixos, N., Moltó, E., Gómez-Sanchis, J., & Blasco, J. (2011). Advances in machine vision applications for automatic inspection and quality evaluation of fruits and vegetables. *Food and Bioprocess Technology*, 4, 487-504.
- Cunha, L. C., Teixeira, G. H. A., Nardini, V., & Walsh, K. (2016). Quality evaluation of intact açai and juçara fruit by means of near infrared spectroscopy. *Postharvest Biology and Technology*, 112, 64–74.
- Della Cara, R. (2005). In calo i consumi e l'export de pesche e nettarine italiane. *Rivista di Frutticoltura* 7–8, 19–20.
- Downey, G. (1997). Authentication of food and food ingredients by near infrared spectroscopy. *Journal of Near Infrared Spectroscopy* 4, 47–61.
- Eskin, N.A.M., Hoehn, E., Shahidi, F., (2013). Fruits and vegetables, Eskin, N.A.M., Shahidi, F. (Eds.), *Biochemistry of foods*, 49–126.
- Faber, N.M. (1999). Multivariate Sensitivity for the Interpretation of the Effect of Spectral Pretreatment Methods on Near-Infrared Calibration Model Predictions. *Anal. Chem.* (71), 557-565.
- Fang, L., Li, H., Liu, Z., Xian, X. Online evaluation of yellow peach quality by visible and near-infrared Spectroscopy. (2013). *Advance Journal of Food Science and Technology*, 5 (5), pp. 606-612.
- Ferrer, P., Montesinos, J.L., Valero, F. & Solá, C. (2001). Production of native and recombinant lipases by *Candida rugosa*. *Applied Biochemistry and Biotechnology*, vol. 95, no. 3, p. 221-255.
- Font, D., Tresanchez, M., Pallejà, T., Teixidó, M., Martinez, D., Moreno, J. & Palacín, J. (2014). An image processing method for in-line nectarine variety verification based on the comparison of skin feature histogram vectors. *Computers and Electronics in Agriculture*, 102, 112-119.
- Fu, X., Yibin, Y., Lu, H., Xu, H. & Yu, H. (2007). FT-NIR diffuse reflectance spectroscopy for kiwifruit firmness detection. *Sens. Instrumen. Food Qual.* 1, 29–35.
- GenCat: Generalitat de Catalunya. Technical report 1/2011 and Technical Indicator A2. <<http://www20.gencat.cat>> (accessed 13.05.13).
- Ghiani, A., Negrini, N., Morgutti, S., Baldin, F., Nocito, F. F., Spinardi, A., Mignani, I., Bassi, D. & Cocucci, M. (2011). Melting of 'Big Top' Nectarine Fruit: Some Physiological, Biochemical, and Molecular Aspects. *J. Amer. Soc. Hort. Sci.*, 136, 61-68.
- Golic, M., Walsh, K.B. Robustness of calibration models based on near infrared spectroscopy for the in-line grading of stonefruit for total soluble solids content. (2006) *Analytica Chimica Acta*, 555 (2), pp. 286-291.

- Gómez, A.H., He, Y. & Pereira, A.G. (2006). Nondestructive measurement of acidity: Soluble solids and firmness of Satsuma mandarin using Vis/NIRspectroscopy techniques. *J. Food Eng.*, 77(2), 313-319.
- Gorry, P. A. (1990). General least-squares smoothing and differentiation by the convolution (Savitzky-Golay) method. *Analytical Chemistry*, 62, 570-573.
- He, Y., Li, X.L. & Shao, Y.N. (2006). Discrimination of varieties of apple using near infrared spectra based on principal component analysis and artificial neural network model. *Spectroscopy and Spectral Analysis*, 26, 850–853.
- Hernández, A., He, Y. & García, A. (2006). Non-destructive measurement of acidity, soluble solids and firmness of Satsuma mandarin using Vis/NIR-spectroscopy techniques. *Journal of Food Engineering* 77, 313-319.
- Huang, H., Yu, H., Xu, H. & Ying, Y. (2008). Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: A review. *Journal of Food Engineering*, 87 (3), 303-313.
- Huang, L., Wu, D., Jin, H., Zhang, J., He, Y. & Lou, C. (2011). Internal quality determination of fruit with bumpy surface using visible and near infrared spectroscopy and chemometrics: A case study with mulberry fruit. *Biosystems Engineering* 109 (4), 377-384.
- Iglesias, I. & Echeverría, G. (2009). Differential effect of cultivar and harvest date on nectarine colour, quality and consumer acceptance. *Scientia Horticulturae*, 120, 41-50.
- Iglesias, I. (2013). Peach production in Spain: Current situation and trends, from production to consumption. *Proceedings of the 4th Conference, Innovation in fruit growing, Blegrade*, 75-89.
- Jaiswal, P., Jha, S.N. & Bharadwaj, R. (2012). Non-destructive prediction of quality of intact banana using spectroscopy. *Scientia Horticulturae* 135, 14-22.
- Kamruzzaman, M., ElMasry, G., Sun, D. & Allen, P. (2012). Non-destructive prediction and visualization of chemical composition in lamb meat using NIR hyperspectral imaging and multivariate regression. *Innovate Food Science and Emerging Technologies*, 16, 218-226.
- Kozak, M. & Scaman, C. H. (2008). Unsupervised classification methods in food sciences: discussion and outlook. *Journal of the Science of Food and Agriculture*, 88, 1115-1127.
- Lichtenthaler, H.K. & Buschmann, C. (2001). Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. *Curr. Prot. Food Anal. Chem.* (F:F4:F4.3.).
- Liu, Y., Chen, X., & Ouyang, A. (2008). Nondestructive determination of pear internal quality indices by visible and near infrared spectrometry. *LWT - Food Science and Technology*, 41, 1720–1725.

- Lorente, D., Aleixos, N., Gómez-Sanchis, J., Cubero, S., García-Navarrete, O.L., & Blasco, J., (2012). Recent advances and applications of hyperspectral imaging for fruit and vegetable quality assessment. *Food Bioprocess Technology* 5, 1121–1142.
- Lorente, D., Escandell-Montero, P., Cubero, S., Gómez-Sanchis, J. & Blasco, J. (2015). Visible-NIR reflectance spectroscopy and manifold learning methods applied to the detection of fungal infections on citrus fruit. *Journal of Food Engineering*, 163, 17-21.
- Lu, R. (2004). Multispectral imaging for predicting firmness and soluble solids content of apple fruit. *Postharvest Biology and Technology* 31 (2), 147- 157.
- Ma, G., Fu, X.-P., Zhou, Y., Ying, Y.-B., Xu, H.-R., Xie, L.-J., Lin, T. Nondestructive sugar content determination of peaches by using near infrared spectroscopy technique. (2007) *Guang Pu Xue Yu Guang Pu Fen Xi/Spectroscopy and Spectral Analysis*, 27 (5), pp. 907-910.
- Magwaza, L. S., Opara, L. U., Nieuwoudt, H., Cronje, P. J. R., Saeys, W. & Nicolai, B. (2012). NIR Spectroscopy Applications for Internal and External Quality Analysis of Citrus Fruit- A Review. *Food Bioprocess Technol*, 5, 425-444.
- Martens, H., Nielsen, J. P. & Engelsen, S. B. (2003). Light scattering and light absorbance separated by extended multiplicative signal correction. Application to near-infrared transmission analysis of powder mixtures. *Anal. Chem.* 75, 394-404.
- Martins, P. A., Cirino de Carvalho, L., Cunha, L. C., Manhas, F. & Teixeira, G. H. (2016). Robust PLS models for soluble solids content and firmness determination in low chilling peach using near infrared spectroscopy (NIR). *Postharvest Biology and Technology*, 111, 345-351.
- McGlone, V. A., & Kawano, S. (1998). Firmness, dry-matter and soluble-solids assessment of post-harvest kiwifruit by NIR spectroscopy. *Postharvest Biology and Technology*, 13, 131–141.
- Merzlyak, M. N., Solo, A. E., & Gitelson, A. A. (2003). Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. *Postharvest Biology and Technology*, 27, 197–211.
- Nicolai, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, I.K., Lammertyn, J. (2007). Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: a review. *Postharvest Biol. Technol.* 46, 99–118.
- Osborne, B.G., Fearn, T., Hindle, P.H. (1993). *Practical NIR Spectroscopy with Applications in Food and Beverage Analysis*, 2nd ed. Longman Group, Burnt Mill, Harlow, Essex, England, UK, pp. 123–132.

- Padilla-Zakour, O. I. (2009). Good manufacturing practices. In N. Heredia, I. Wesley, & S. Garcia (Eds.), *Microbiologically Safe Foods*, 395 – 415. New York: John Wiley and Sons Inc.
- Peiris, K.H.S., Dull, G.G., Leffler, R.G., Kays, S.J. Near-infrared spectrometric method for nondestructive determination of soluble solids content of peaches. (1998) *Journal of the American Society for Horticultural Science*, 123 (5), pp. 898-905.
- Pérez-Marín, D., Sánchez, M. T., Paz, P., González-Dugo, V. & Soriano, M. A. (2011). Postharvest shelf-life discrimination of nectarines produced under different irrigation strategies using NIR-spectroscopy. *Food Science and Technology*, 44, 1405-1414.
- Ravaglia, G., Sansavini, S., Ventura, M., Tabanelli, D. (1996). Indici di maturazione e miglioramento qualitativo delle pesche. *Revista di Frutticoltura* 3, 61–66.
- Reita, G., Peano, C., Saranwong, S. & Kawano, S. (2008). An evaluating technique for variety compatibility of fruit applied to a near infrared Birx calibration system: a case study using Brix calibration for nectarines. *Journal of Near Infrared Spectroscopy*, 16 (2), 83–89.
- Rodriguez-Campos, J., Escalona-Buendía, H. B., Orozco-Avila, I., Lugo-Cervantes, E. & Jaramillo-Flores, M. E. (2011). Dynamics of volatile and non-volatile compounds in cocoa (*Theobroma cacao* L.) during fermentation and drying processes using principal components analysis. *Food Research International*, 44, 250–258.
- Sádecká, J., Jakubíková, M., Májek, P. & Kleinová, A. (2016). Classification of plum spirit drinks by synchronous fluorescence spectroscopy. *Food Chemistry*, 196, 783–790
- Sánchez, M. T., De la Haba, M. J., Guerrero, J. E., Garrido-Varo, A., & Pérez-Marín, D. (2011). Testing of a local approach for the prediction of quality parameters in intact nectarines using a portable NIRS instrument. *Postharvest Biology and Technology*, 60(2), 130–135.
- Santos, P., Santos, F., Santos, J. & Bezerra, H. (2013). Application of extended multiplicative signal correction to short-wavelength near infrared spectra of moisture in Marzipan. *Journal of Data Analysis and Information Processing*, 1, 30-34.
- Savitzky, A., & Golay, M. J. E. (1964). Smoothing and differentiation of data by simplified squares procedures. *Analytical Chemistry*, 36, 1627-1639.
- Shao, Y., He, Y., Gómez, A.H., Pereir, A.G., Qiu, Z., Zhang, Y. (2007). Visible/near infrared spectrometric technique for nondestructive assessment of tomato ‘Heatwave’ (*Lycopersicon esculentum*) quality characteristics. *J. Food Eng.* 81 (4), 672–678.

- Singh, Z., Singh, R.K., Sane, V.A. & Nath, P. (2013). Mango–Postharvest biology and biotechnology. *Crit. Rev. Plant Sci.* 32 (4), 217–236.
- Soares, S. F. C., Gomes, A. A., Galvão Filho, A. R., Araújo, M. C. U., & Galvão, R. K. H. (2013). The successive projections algorithm. *Trends in Analytical Chemistry*, 42, 84–98.
- Tijsskens, L. M. M., Zerbini, P. E., Schouten, R. E., Vanoli, M., Jacob, S., Grassi, M., Torricelli, A. (2007). Assessing harvest maturity in nectarines. *Postharvest Biology and Technology*, 45, 204–213.
- Valero, A., Marín, S., Ramos, A. J., & Sanchis, V. (2007). Effect of preharvest fungicides and interacting fungi on *Aspergillus carbonarius* growth and ochratoxin A synthesis in dehydrating grapes. *Letters in Applied Microbiology*, 45, 194–199.
- Walsh, K.B., Golic, M. & Greensill, C.V. (2004). Sorting of fruit and vegetables using near infrared spectroscopy: application to soluble solids and dry matter content. *J. Near Infrared Spectrosc.* 12, 141–148.
- Williams, P. C. & Norris, K. H. (1987). Qualitative applications of near infrared reflectance spectroscopy. In P. C. Williams & K. H. Norris (Eds.), *Near infrared technology in the agricultural and food industries*, 241–246.

Figure Captions

Figure 1. A labelled photograph of the VIS-NIR equipment.

Figure 2. VIS-NIR spectra of the 150 cv. 'Big Top' samples; a) Raw and b) Smoothing Savitzky-Golay + EMSC transformed.

Figure 3. Mean and standard deviation of a) firmness and b) TSS of nectarines at different sets of analysis. Discontinuous lines in the mechanical plot (left) indicate firmness thresholds.

Figure 4. a) b) and c) Flesh colour attributes (L^* , h^* and C^*); and d) an example of internal colour appearance of nectarines cv. 'Big Top'; and e) cv. 'Magique' during the storage period.

Figure 5. Evolution of the IQI during the storage period of the a) nectarines cv. 'Big Top' and b) nectarines cv. 'Magique'.

Figure 6. Fruit samples absorbance spectra between 360 and 1795 nm for the two varieties of nectarines at different storage times.

Figure 7. Predicted vs measured values of IQI in the examined samples of variety a) 'Big Top' and b) 'Magique'.

Figure 8. Projection of nectarines samples in the space defined by the two first PCs.

Figure 9. Estimated class values for training and validation sets for varietal discrimination by PLS-DA model.

Figure 10. Discrimination plot of the LDA results for the validation samples.

Table Captions

Table 1. Results of the PLS models for the prediction of the IQI in nectarines samples with different flesh colour.

Table 2. Confusion matrix obtained in prediction for the PLS-DA and LDA analysis.

Figure 1

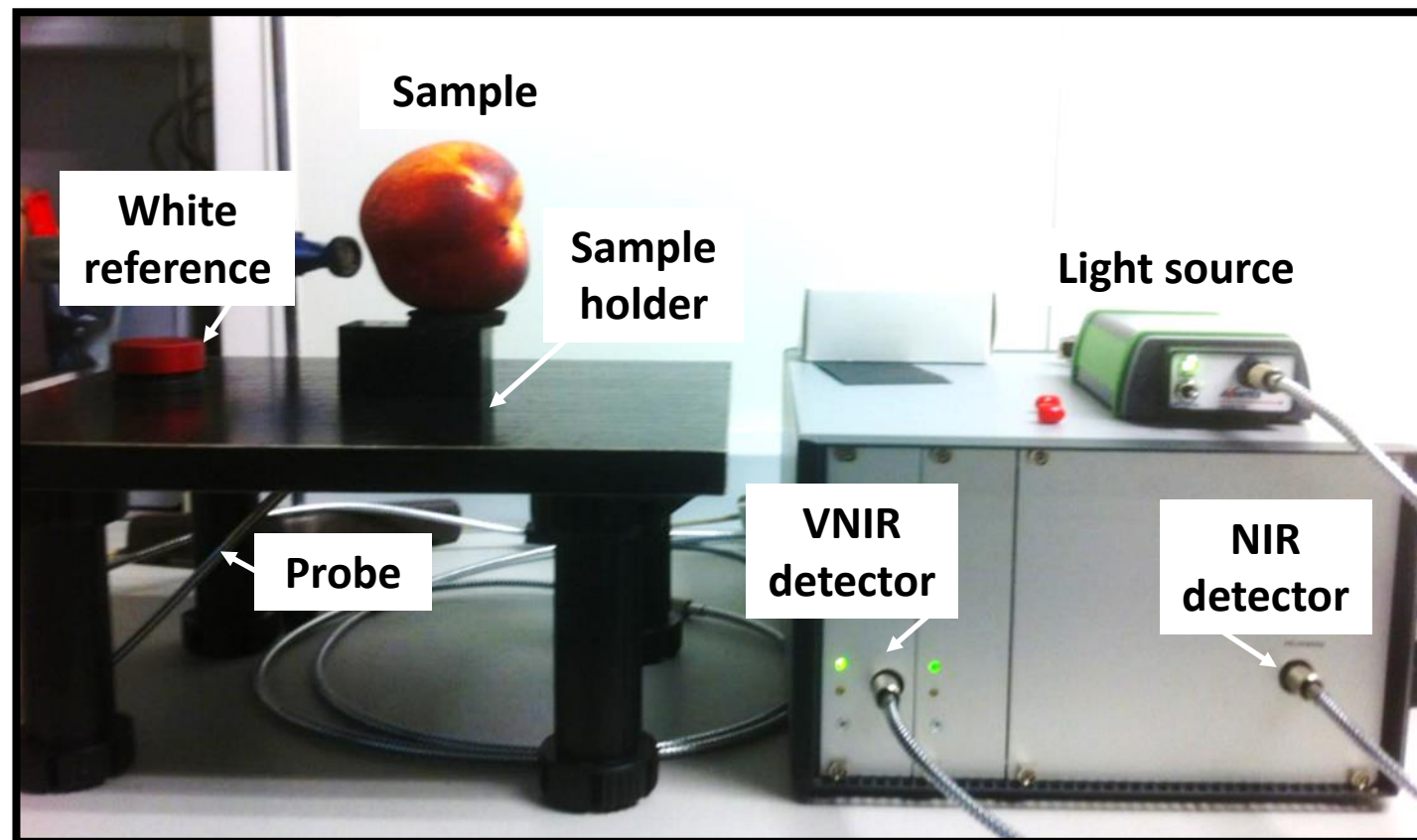


Figure 2

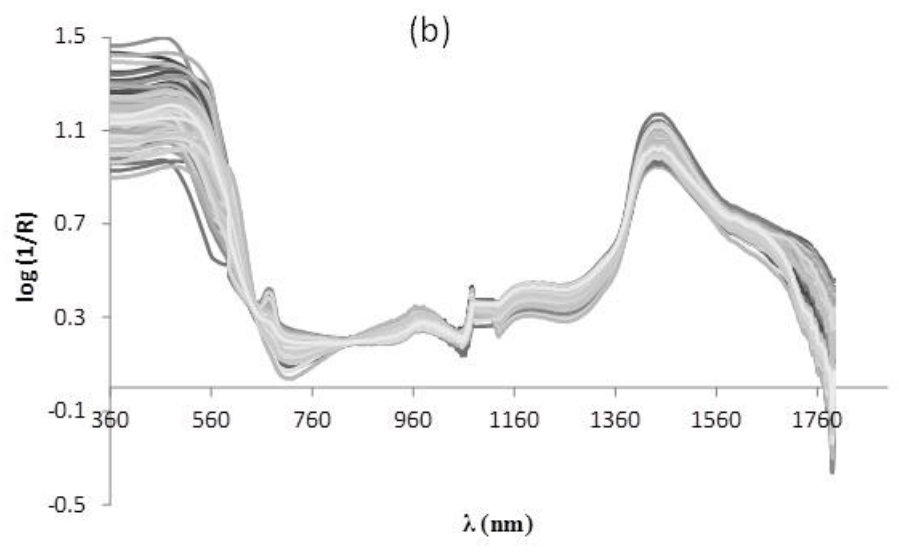
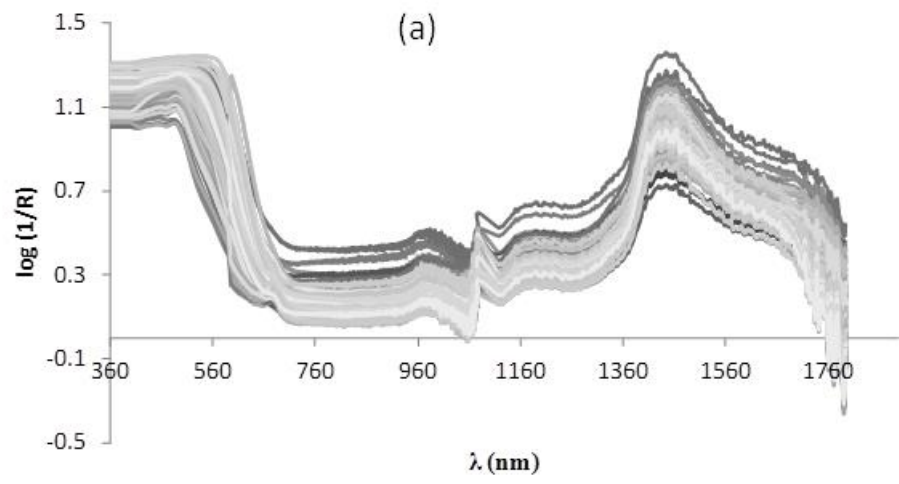


Figure 3

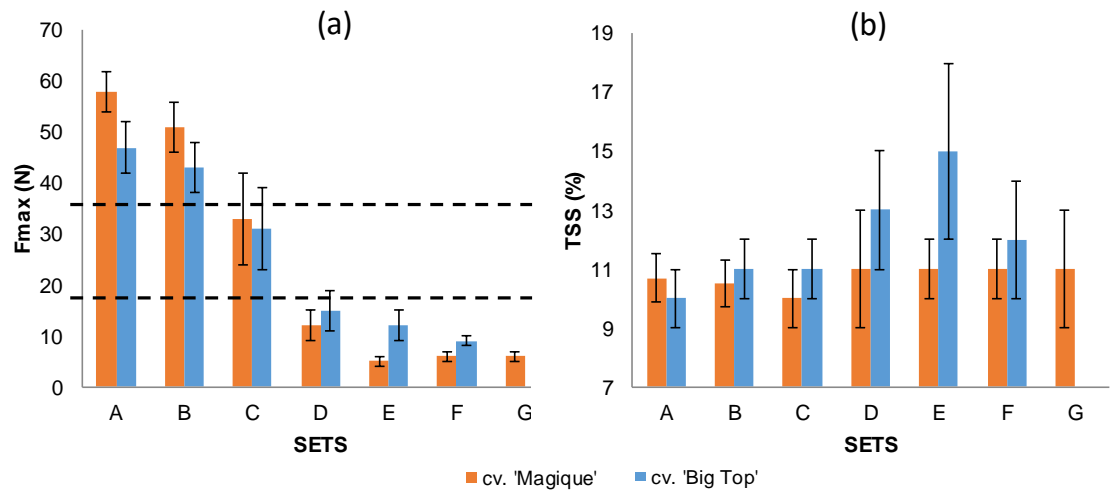


Figure 4

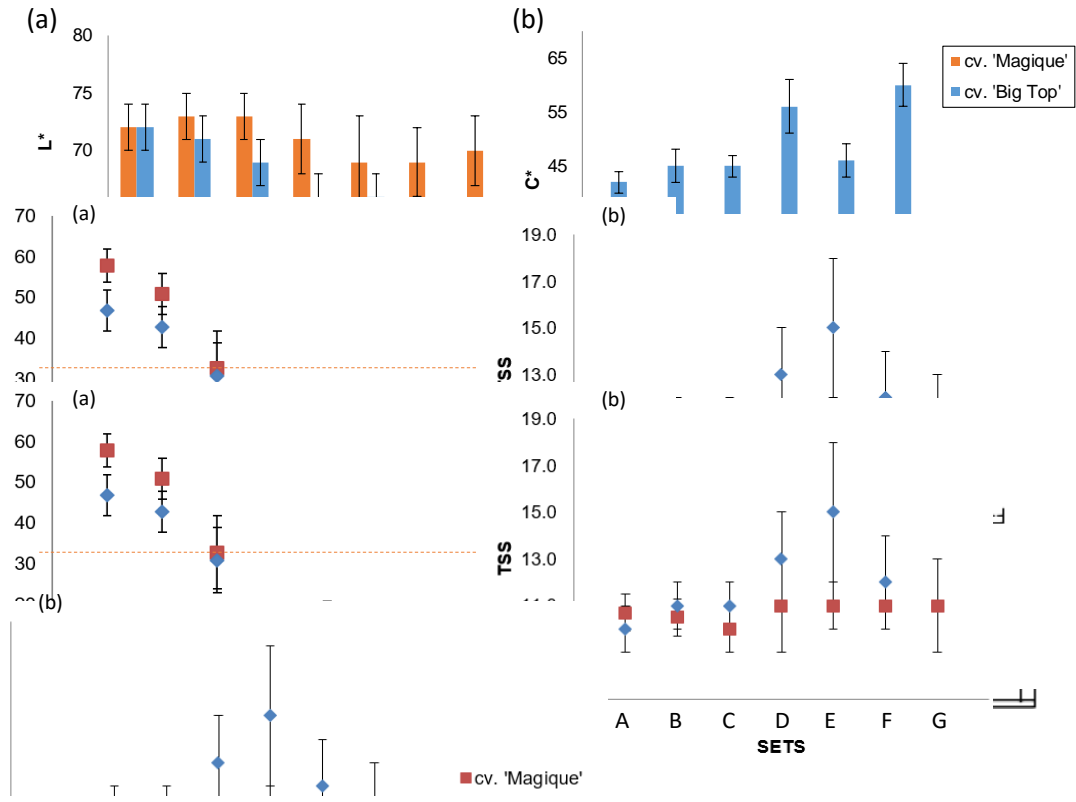


Figure 5

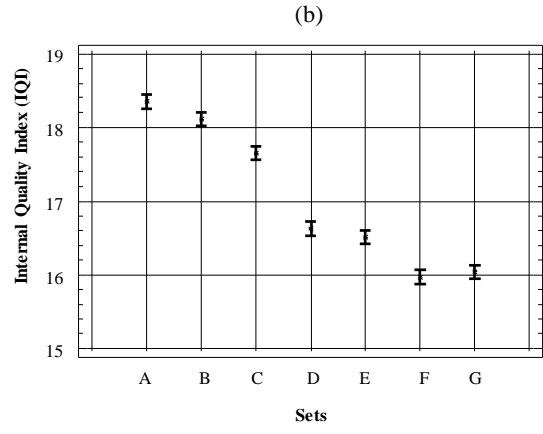
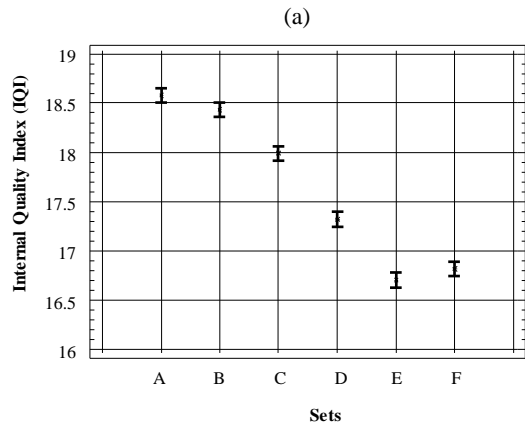


Figure 6

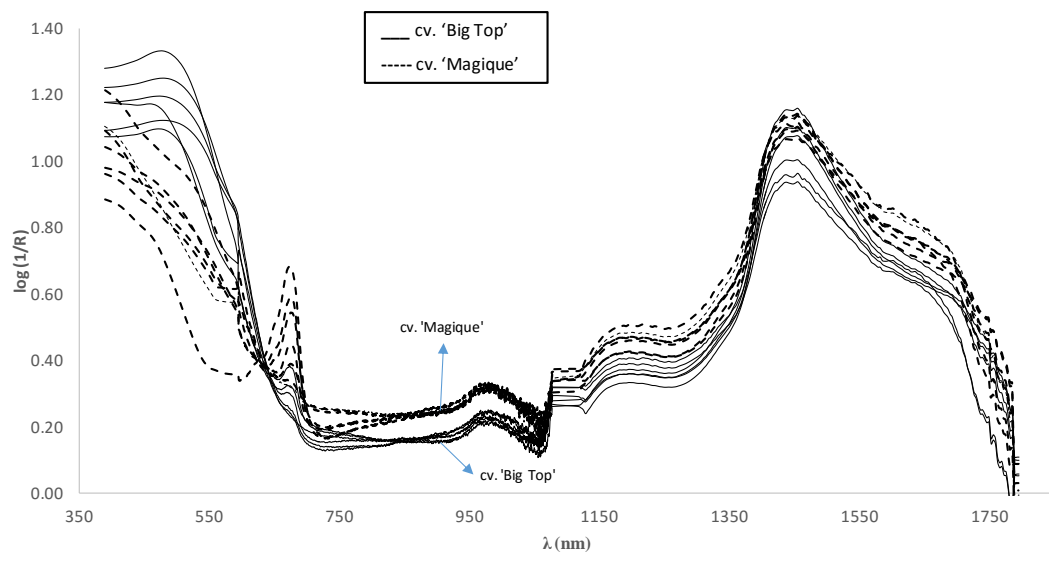


Figure 7

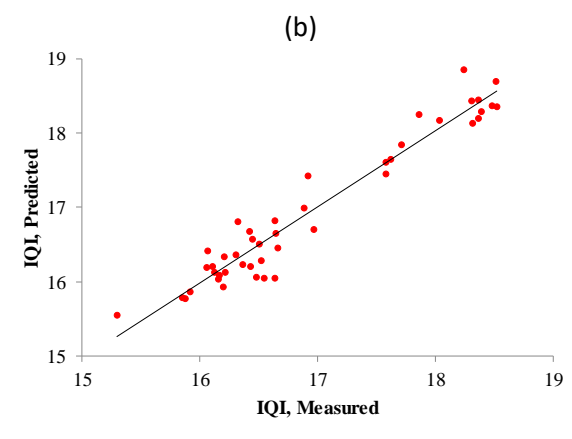
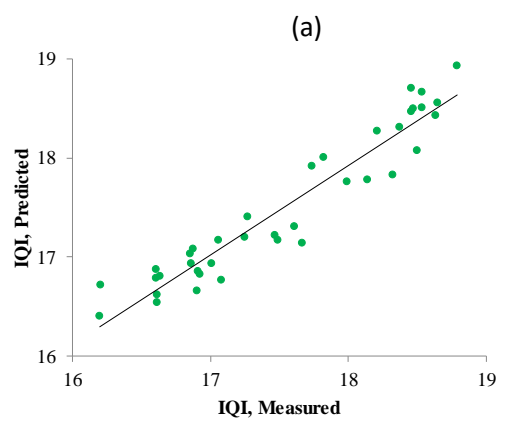


Figure 8

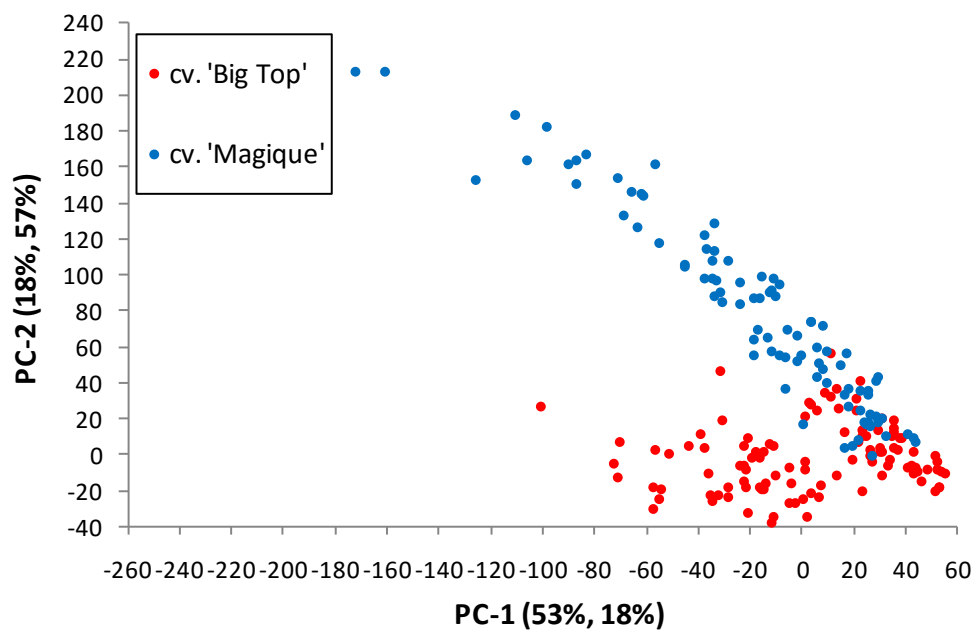


Figure 9

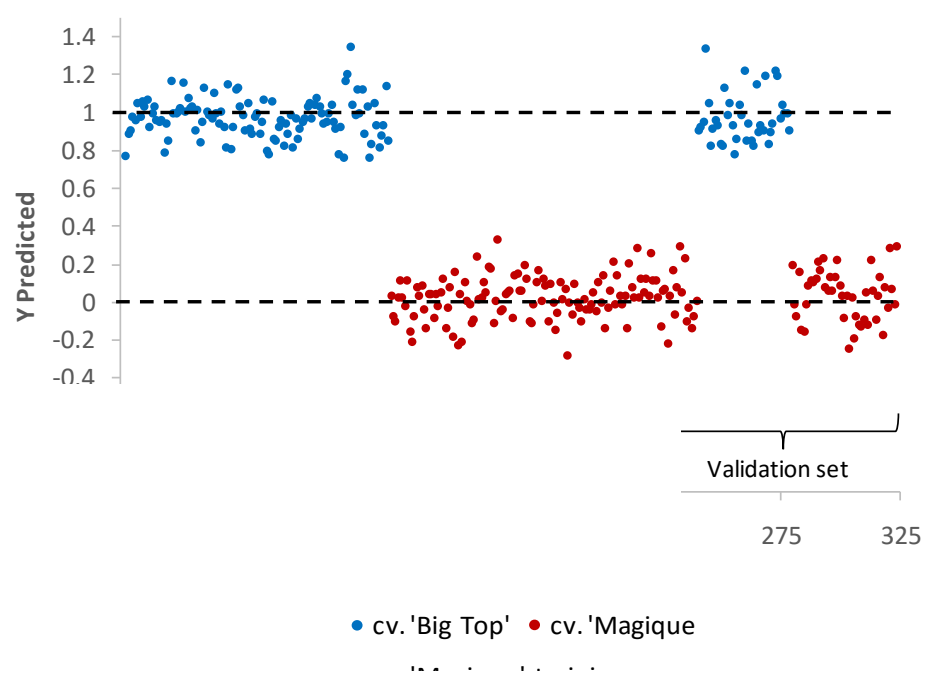


Figure 10

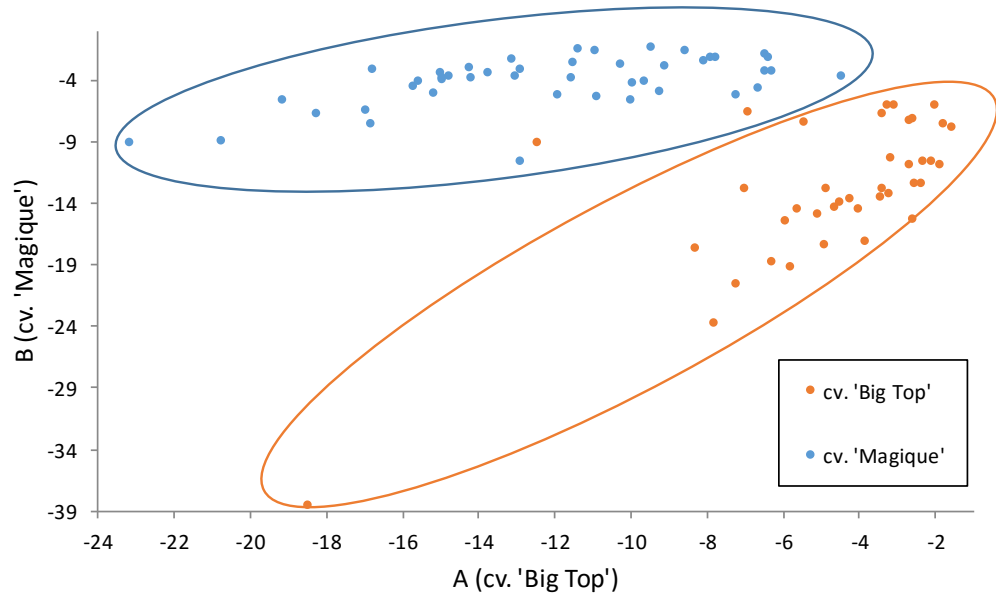


Table 1. Results of the PLS models for the prediction of the IQI in nectarines samples with different flesh colour.

Parameter	#LV	Calibration		Cross Validation		Prediction	
		R^2_c	RMSEC	R^2_{cv}	RMSECV	R^2_p	RMSEP
IQI, cv. 'Big Top'	7	0.948	0.179	0.910	0.237	0.909	0.235
IQI, cv. 'Magique'	7	0.965	0.183	0.942	0.238	0.927	0.238

Table 2. Confusion matrix obtained in prediction for the PLS-DA and LDA analysis

		Reference variety		Classification accuracy	
		cv. 'Big Top'	cv. 'Magique'		
LDA	Sample variety	cv. 'Big Top'	94.87% (37)	5.13% (2)	97.44%
		cv. 'Magique'	0% (0)	100% (45)	
PLS-DA	Sample variety	cv. 'Big Top'	100% (39)	0% (0)	100%
		cv. 'Magique'	0% (0)	100% (45)	