Internal and external quality assessment of mandarins on-tree and at harvest using a portable NIR spectrophotometer

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Abstract

Citrus growers are increasingly demanding rapid, cost-effective, green, and non-destructive methods for monitoring changes in physical-chemical quality during on-tree ripening, with a view to establishing the optimum harvest date. This study sought to evaluate the performance of a handheld MEMS-based NIR spectrophotometer, used in conjunction with chemometric techniques, for the prediction of changes not only in major external quality parameters such as weight, size (equatorial and axial diameters) and color (L*, a*, b*, C*, h*, color index), but also in internal physical parameters (firmness, maximum penetration force, pericarp thickness, juice weight, juice content) and chemical properties (soluble solids content, pH, titratable acidity, maturity index) in intact mandarins during the pre-harvest period, as a means of establishing the most suitable harvest date. The models obtained by applying MPLS regression to spectroscopic data yielded promising results for on-tree external quality measurements, particularly for color-related parameters (a*, C* and color index), and for all the internal chemical parameters studied. These results confirm that changes in intact mandarin quality parameters during on-tree ripening can be measured non-invasively using the new generation of portable MEMS-based NIRS sensors, allowing the ripening process to be charted in individual fruits not only in terms of visual appearance, but also in terms of taste- and texture-related features, this being a major step towards the selective harvesting of citrus fruits depending on their final destination.

Keywords: NIR spectroscopy, Intact mandarin, Quality parameters, Portable sensor, MEMS technology, On-tree, At harvest.

1. Introduction

Mandarins include a diverse group of citrus fruits that are characterized by bright colored peel and pulp, excellent flavor, easy-to-peel rind and segments that separate easily. They are usually consumed in raw form or in fruit salads as well as in juice, jam, squash and syrup.

For mandarin producers, the critical decision regarding harvest date is based on perceived fruit ripeness, since the ripeness of harvested fruit has a major impact on its shelf life, quality and market price. Hence, certain standards of ripeness must be borne in mind at harvesting. The measure most widely used to assess mandarin ripeness is peel color: fruits are considered ripe if they are orange in color over 75% or more of their surface. However, mandarin harvesting quality also depends on soluble solids content (SSC) and the acidity of the juice, which should have a SSC of 8.5% or more (Zude et al., 2008; Liu et al., 2010; Antonucci et al., 2011).

At present, the charting of on-tree ripening with a view to establishing the optimum harvesting date is based purely on the measurement of external color together with occasional destructive measurement of internal quality parameters. In most cases, however, the harvesting date is established only on the basis of total SSC, as measured by refractometry (Zude et al., 2008).

Therefore, both the mandarin industry and the final consumer would benefit greatly from the incorporation of non-destructive technology for the on-tree measurement of both internal and external quality parameters, which would facilitate real-time decision-making in the field.

Near-infrared spectroscopy (NIRS) may provide an ideal way of meeting industry needs: it is a non-destructive technique that combines swift and precise measurement with considerable versatility, high throughput and low cost. Moreover, the current trend

towards the use of NIRS technology *in situ* has led to the miniaturization of optical components and the application of new techniques such as digital transform spectroscopy (DTS), which have enabled the development of compact, portable NIRS instruments ideal for use in the field; they can either be hand-held or mounted on tractors.

Portable NIRS equipment based on MEMS (micro-electro-mechanical system) technology offers considerable advantages in terms of instrument size and robustness, spectral range and low manufacturing cost (Geller, 2007). Yet although several hand-held, highly-portable MEMS instruments are currently being marketed, only a limited amount of scientific information is available regarding NIRS-MEMS applications in the agrofood sector; to date, studies have been limited to nectarines (Pérez-Marín et al., 2009, 2010, 2011; Sánchez et al., 2011), strawberries (Sánchez et al., 2012), and grapes (González-Caballero et al., 2012).

There are no reports in the literature regarding the use of MEMS instruments for the pre-harvest monitoring of mandarins with a view to establishing the optimum time for harvesting, since research to date on the use of NIRS technology for mandarin quality control has focused on the measurement of SSC using diode-array instruments (Kawano et al., 1993; Greensill and Walsh, 2002; McGlone et al., 2003; Walsh et al., 2004; Guthrie et al., 2005a, b; Xudong et al., 2009; Antonucci et al., 2011) and monochromators (Miyamoto and Kitano, 1995; Tsuchikawa et al., 2003; Hernández-Gómez et al. 2006). Several authors have measured acidity-related parameters (titratable acidity and pH), again using diode-array (McGlone et al., 2003; Xudong et al., 2009; Liu et al., 2010; Antonucci et al., 2011) and monochromator instruments (Miyamoto and Kitano, 1995; Miyamoto et al., 1998; Tsuchikawa et al., 2003; Hernández-Gómez et al. 2006). A monochromator has also been used to measure firmness (penetration force)

in mandarins (Hernández-Gómez et al., 2006), while Xudong et al. (2009) used a diode-array instrument to measure surface color.

The present study sought to assess the feasibility of using a low-cost miniaturized, handheld, near-infrared device based on MEMS technology in intact mandarins as a means of characterizing external and internal variations in on-tree ripening, with a view to optimizing harvesting times and thus enabling selective harvesting.

2. Materials and Methods

2.1. Mandarin samples

The initial sample set comprised 256 mandarins (cv. 'Clemevilla') grown on a commercial plantation near the village of La Campana (Seville, Spain) under four different irrigation regimes.

Each experimental plot comprised 3 rows of four trees, with four repetitions for each irrigation regime; mandarins were monitored on the two central trees in each plot. Thus ripening was monitored on 8 trees for each of the 4 irrigation regimes, giving a total of 32 trees.

A total of 8 mandarins were labeled on each of the 32 trees: one for each of the four possible orientations (north, south, east and west) and one for each of two heights on the tree (1.25 and 1.75 m), thus giving a total of 256 mandarins. However, in the course of the study, 22 mandarins dropped off the tree, and were therefore excluded. The final sample set thus comprised 234 mandarins.

Mandarins were harvested on six different dates in 2010: October 29 (32 mandarins), November 5 (32 mandarins), November 12 (31 mandarins; one ripe orange dropped off the tree, and was thus excluded), November 19 (34 mandarins = 32 plus 2 due to be picked on December 3, which dropped off during harvesting of the earlier batch), December 3 (30 mandarins) and finally December 16, by which time

commercial ripeness had been attained (75 mandarins). Although the initial intention was to monitor ripening by picking fruit every 7 days until commercial ripeness was attained, no fruit could be picked on November 26 and December 9, since bad weather rendered the plantation inaccessible.

For the first harvesting date, mandarins were selected using a pre-arranged strategy: for the first 4 weeks of the study, mandarins growing at a height of 1.75 m from the ground were picked, taking fruit from each of the four orientations: west-facing (first week); south-facing (second week), east-facing (third week), and north-facing (fourth week) for each of the irrigation regimes under study. In the fifth week (December 3) west-facing mandarins growing at 1.25 m from the ground were picked for all irrigation regimes studied; finally, on December 16 (commercial ripeness) north-, south- and east-facing mandarins were picked for each of the irrigation regimes.

Harvested mandarins were kept in refrigerated storage at 5ºC and 90% RH until the following day, when laboratory testing was performed. Prior to each test, mandarins were allowed to reach room temperature. All tests were performed at 20ºC.

2.2. Spectral data acquisition

NIR spectra of intact mandarins were collected in reflectance mode (log 1/R) using a handheld micro-electromechanical system (MEMS) instrument (Phazir 2400, Polychromix, Inc., Wilmington, MA, USA).

The Phazir 2400 is an integrated near-infrared handheld analyzer that incorporates all the essential components to deliver on-tree applications. The spectrophotometer operates between 1600 and 2400 nm with an 8 nm sampling interval (pixel resolution 8 nm, optical resolution 12 nm). Four spectral measurements were made on each mandarin whilst on the tree, taking orientation (north, south, east, and west) into account. The four spectra were averaged to provide a mean spectrum for each fruit.

2.3. Measurement of physical-chemical parameters

2.3.1. External quality parameters

Mandarins were individually weighed on an electronic balance (0-1.000 ± 0.01 g; model P1000 N, Metter-Toledo, GmbH, Greifensee, Switzerland).

Equatorial and axial diameters were then measured using a digital precision calibrator (0-300 ± 0.01 mm; Comecta, Barcelona, Spain).

Skin or external color values (L*, a*, b*) were individually measured at the equator, turning the fruit through 90º between measurements, using a Minolta Chroma Meter CR-400 (Minolta Corporation, Ramsay, NJ, USA). Chroma (C*), hue angle (h*) and color index were calculated as (a*^2+b*^2)^(1/2), $\tan^{-1}$ (b*/a*) and (1000a*)/(L*b*), respectively. Illuminant C and 2-degree standard observer measurements were made in all cases. The four measurements obtained per fruit for each of the color parameters tested were averaged.

2.3.2. Internal quality parameters

Firmness was measured as the maximum force required to penetrate the mandarins to a puncturing depth of 10 mm. The maximum force required to pierce the fruit after a total penetration of 15 mm was also established. In both cases, a 6-mm cylindrical tip was used. Mandarins were arranged with the stem-calyx axis horizontal; the first measurement was made at a point on the equator, and the second after turning the mandarin through 180º. Texture measurements were made using a Universal Instron Texturometer (Model 3343, single-column, Instron Corporation, Norwood, MA, USA), with a head speed of 0.0016 m/s (100 mm/min) and a 1000 N load cell.

Fruits were then halved through the equatorial plane, and pericarp thickness was measured at two points on one of the halves using the same digital calibrator. Fruits were then individually pressed using a domestic juicer, and the juice obtained was

weighed on an electronic balance (0–210 ± 0.001 g; model C-600-SX, Cobos, Barcelona, Spain); the juice weight/fruit weight ratio was also calculated.

Soluble solids content (SSC), pH and titratable acidity (TA) were measured following Obenland et al. (2008). The maturity index (SSC:TA ratio) was also calculated.

All external quality parameters (weight, axial and equatorial diameters and color) and internal physical quality parameters (firmness, maximum penetration force and pericarp thickness) were measured in all 234 harvested mandarins.

For juice weight and juice content, measurements were made in only 233 mandarins, since one of the fruits harvested in the first week was unripe (completely green), and juice extraction was impossible. Soluble solids content (SSC) was measured in 231 mandarins, since the other three presented filtering problems rendering it impossible to extract clear juice. Measurements of pH and titratable acidity were made in 223 and 206 mandarins, respectively, due to the difficulty in obtaining sufficiently clear liquid from fruit harvested during the first weeks (Obeland, 2008). The maturity index (SSC:TA ratio) was calculated for 206 fruits.

All samples were analyzed in duplicate.

2.4. Data analysis: definition of calibration and validation sets

Dixon's test ($p < 0.05$), as incorporated in the Statgraphics Centurion XV software package (StatPoint, Inc., Warrenton, Northern Virgina, USA), was used to exclude samples considered to be outliers in physical-chemical terms. Of the 234 mandarins analyzed, the final sample set comprised 214 for all the parameters analyzed, except for juice weight and juice content (N = 213 samples), SSC (N = 211 samples), pH (N = 206 samples) and for tritatable acidity and maturity index (N = 190 samples).

Next, and prior to carrying out NIRS calibrations, the CENTER algorithm included in the WinISI II software package ver. 1.50 (Infrasoft International LLC, Port Matilda, PA, USA) was applied to ensure a structured population selection based solely on spectral information for the establishment of calibration and validation sets (Shenk and Westerhaus 1991a, b; 1995a).

The CENTER algorithm was applied in the spectral region 1600-2400 nm. Mathematical treatments SNV (Standard Normal Variate) and DT (De-trending) were applied for scatter correction (Barnes et al., 1989), together with the mathematical derivation treatment '1,5,5,1', where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing, and the fourth is the second smoothing (Shenk and Westerhaus, 1995b; ISI, 2000).

After elimination of outlier spectra, and having ordered the sample set by spectral distances (from smallest to greatest distance to the center), the samples forming the validation set were selected by taking one sample out of every three in the initial set. After this procedure, the calibration and validation sets thus comprised the samples shown in Table 1.

Data were subjected to chemometric treatment using the WinISI software package (ISI, 2000).

2.5. Data pre-processing and calibration model construction using linear and non-linear regression strategies.

NIR calibration models were initially constructed for the prediction of external and internal quality parameters in intact mandarins using MPLS, a linear regression method (Shenk and Westerhaus, 1995a). Six cross-validation steps were included in the process in order to avoid overfitting (Shenk and Westerhaus, 1995a).

Prior to model development, a number of different pre-processing combinations were evaluated for scatter correction, including SNV and DT. Additionally, a total of four derivative mathematical treatments were tested: 1,5,5,1; 2,5,5,1; 1,10,5,1 and 2,10,5,1.

The statistics used to select the best equations were: the coefficient of determination for calibration (R^2), the standard error of calibration (SEC), the coefficient of determination for cross calibration (r^2), the standard error of cross validation (SECV) and the coefficient of variation (CV), defined as the ratio between SECV and the mean value of the reference data in the calibration set. Furthermore, the Residual Predictive Deviation (RPD) was calculated as the ratio of the standard deviation (SD) of the reference data to the SECV. This statistic, together with the CV, enables SECV to be standardized, facilitating the comparison of results obtained with sets of different means (Williams, 2001).

The best predictive models obtained, selected by statistical criteria, were subsequently subjected to external validation following the protocol outlined by Shenk et al. (2001). Generally speaking, for calibration sets comprising 100 or more samples and validation sets composed of nine of more samples, the following control limits can be assumed: SEP(c) should not exceed 1.30 times the SEC and bias should not exceed ±0.6 times the SEC; minimum r^2 = 0.60 and minimum slope 0.90.

2.6. Statistical analysis

Measured characteristics were analyzed by one-way analysis of variance (ANOVA) with harvest day as variable. Significantly different means were separated by the least significant difference at the 5% level. All data were analyzed using the Statgraphics Centurion XV software package (StatPoint Inc., Warrenton, Northern Virginia, USA).

3. Results and Discussion

3.1. Changes in quality parameters during on-tree ripening

The changes observed in the external and internal physical–chemical properties of mandarins during on-tree ripening are shown in Table 1. As expected, changes in the different parameters generally reflected the physiological development of the fruit.

Broadly speaking, fruit size (weight, equatorial diameter), color-related parameters (a*, C* and color index), juice weight, juice content, SSC, pH and maturity index increased, while firmness, maximum penetration force and titratable acidity decreased during on-tree ripening through the six harvest dates analyzed.

Fruit weight and equatorial diameter increased constantly during ripening, attaining an overall increase of 44% and 34%, respectively, by the end of the harvest period studied. With regard to axial diameter, it should be noted that while values on the first harvest date were significantly higher ($p < 0.05$) than those recorded on later dates, detailed analysis revealed a significant increase ($p < 0.05$) from the second (52.64 mm) through to the sixth harvest day (56.36 mm).

Values for color parameters and luminosity (L*) on the first harvest date (height 1.75 m, west-facing) were significantly lower ($p < 0.05$) than those recorded for fruit harvested later; mandarins picked on the second, third and fourth harvest dates (height 1.75 m, south-, east- and north-facing, respectively) displayed the highest values for L*, suggesting that skin luminosity diminished as harvesting progressed. Fruit picked on the fifth and sixth harvest dates (1.25 m) exhibited L* values significantly higher than those obtained on the first harvest day, but lower than those recorded for fruit harvested at 1.75 m, thus highlighting the influence of height-on-tree on ripening.

Results for parameter a* (green-red) increased significantly during the harvesting period. Fruit was initially green, and gradually acquired red coloring in the course of

ripening. Similarly, values for parameter b* (blue-yellow) rose significantly over the course of the study, fruit displaying higher positive values (yellow) as ripening progressed; higher-placed fruits also recorded significantly higher values ($p < 0.05$) for this parameter.

These changes in a* and b* during ripening are due mainly to the conversion of chloroplasts to chromoplasts, and to an increase in carotenoid levels which give the mandarin skin its characteristic color (García-Luis et al., 2002).

Color saturation (C*) and the color index increased significantly during ripening, due also to higher carotenoid levels. By contrast, values for the parameter h* rose significantly ($p < 0.05$) from the first harvest day to the second, but thereafter displayed no significant change ($p > 0.05$). These changes in mandarin skin color, from green-yellowish tones (negative a* and positive b*) to orange-reddish tones (positive a* and b*) typically occur in the course of on-tree ripening.

Firmness and maximum penetration force declined significantly throughout ripening, each parameter decreasing by around 50% from the first harvest day (13.77 N and 52.57 N, respectively) to the last (6.78 N and 27.89 N, respectively).

Pericarp thickness remained fairly constant throughout the harvesting period, except for the fifth harvest day (December 3) when values were significantly higher (4.09 mm).

Juice weight rose significantly ($p < 0.05$) during ripening, increasing by 70% between the start and the end of the study. An equally significant increase was recorded for juice content throughout ripening; however, it should be noted that while juice weight rose considerably over the period, fruit weight also increased significantly, gaining 22% during the harvesting period. Both parameters are of particular interest to

the citrus-fruit industry, minimum juice content for mandarins being set at 33% (Arias and Toledo, 2000).

Mean values for SSC showed a slight but significant increase (6%) during ripening, whereas TA decreased significantly from about 1.33% citric acid at the beginning of the period to about 1.0% citric acid at the end. Since the decrease in TA was accompanied by a slight change in SSC, a 47% increase was recorded in the SSC:TA ratio. As a result, a significant ($p < 0.05$) rise in pH values was detected over the study period.

3.2. Descriptive data for NIR calibrations and validations

Values for range, mean, standard deviation (SD) and coefficient of variation (CV) for each of the parameters analyzed using the calibration and validation sets after application of the CENTER algorithm and removal of spectral outliers are shown in Table 2, together with the number of samples on which each parameter was measured.

It should be stressed that structured selection using only spectral information treatment algorithms such as CENTER proved adequate and useful, since the calibration and validation sets displayed similar values for mean, range and standard deviation for all study parameters, and ranges for the validation set lay within the range recorded for the calibration set.

All parameters except L*, SSC and pH (CV values below 10% for both sets), displayed considerable variation, as evidenced by the CV value; the greatest variability was recorded for h* (CV = 105.96% calibration set; 42.19% validation set), color index (CV = 81.14% calibration set; 58.77% validation set), a* (CV = 66.75% calibration set; 53.76% validation set) and firmness (CV = 64.29% calibration set; 57.96% validation set), mainly reflecting the fact that mandarins were picked at varying stages of ripeness, from totally unripe – green color over virtually the whole surface – to commercial

ripeness. This wide variation in both the calibration and validation sets is reflected in the frequency histograms shown in Fig. 1.

With regard to color parameters (a*, h* and color index), the rapid changes in fruit coloring during ripening (fruits with negative mean a* values were observed only in the first harvest week) gave rise to a clearly right-skewed distribution curve for a* and color index both in calibration and in validation sets (Fig. 1).

For h* using the calibration set, two distinct areas were clearly apparent (Fig. 1), the first (-89.88 < h* <-68.07) containing samples (N = 18) with a greenish-yellow surface (a* = -15.43-(-0.12); b* = 34.89-55.90), and the second (51.74 < h* < 89.70) containing samples (N = 122) with a* values of between 0.32-42.41 and b* values ranging from 52.99 to 71.93, displaying an orange-reddish external skin coloring.

A similar trend was noted for the validation set: 2 samples were located to the left of the histogram, with h* values of -80.70 and -78.12, a* values of -8.10 and -9.16 and b* values of 49.45 and 43.55, respectively, indicative of a greenish-yellow external surface; a total of 69 samples lay to the right of the histogram, with h* values ranging from 52.77 to 88.67, a* values of 1.24 to 41.35 and b* values in the range 51.99- 71.43, indicative of orange-reddish external coloring.

By contrast, the distribution curve of the "firmness" frequency histogram was skewed to the left; over half the mandarins in the calibration and validation sets (N = 76 and 40, respectively) displayed firmness values ranging from 5.01 N to 9.92 N and from 5.07 N to 9.90 N, respectively.

Williams (2001) and Pérez-Marín et al. (2005) note that sample sets for calibration should ideally ensure uniform distribution of composition across the range of the studied parameter in question in order to obtain robust calibrations.

3.3. Calibration for predicting external quality parameters in mandarins

Cross-validation statistics for the best models obtained for the on-tree prediction of weight, axial and equatorial diameters and color (L*, a*, b*, C*, h* and color index) in intact mandarins using the MPLS algorithm are shown in Table 3.

As the table shows, for certain parameters (weight, axial diameter, L*, b*, C*) the best models were obtained using the SNV+DT combination for scatter correction, whilst for others – equatorial diameter, a*, h* and color index – the best models were constructed without using mathematical pretreatments for this purpose.

Fernández-Cabanás et al. (2006) note that the selection of a suitable spectral pre-treatment is by no means an easy issue, given the strong likelihood of several different mathematical transformations being used, whilst Delwiche and Reeves (2004) argue that the best pre-treatment is not known beforehand and for this reason the analyst should search manually for the pre-treatment that produces the lowest residual values. Moreover, these authors note that the decision regarding the pre-treatment to be used in an NIR calibration must ultimately be based on the judgment of the analyst, since it depends to a great extent on the parameter being modeled.

For all tested parameters except h*, the calibration model displaying the greatest predictive capacity was obtained using the first derivative of the spectrum.

As Table 3 shows, the range of values for all parameters except equatorial diameter, a* and color index decreased over ripening with respect to baseline values (Table 2). During the construction of calibration models using MPLS regression, at each stage in cross-validation a number of samples displayed T statistic values greater than 2.5, and were therefore considered outliers. This may be due to several factors, including an insufficient number of similar samples in the calibration set (Naes et al., 2002). It should be noted that the removal of outliers in terms of the T statistic may subsequently influence the external validation of the models obtained, since there will

be samples in the validation set which, for certain parameters, are either under-represented or unrepresented in the final calibration set.

This is particularly relevant in the case of h* for which, in the course of calibration, all samples with a negative value (N = 18), lying to the left of the frequency histogram (Fig. 1) were removed; this also prompted an increase in the mean value, from 46.47 to 63.29, and a narrowing of the range from (-89.88)-89.70 to 51.74-84.01 (Tables 2 and 3, respectively).

As Table 3 shows, good predictive ability (r^2 = 0.76 and 0.74; SECV = 8.18 and 2.62) was recorded for the measurement of a* and color index parameters. Following Williams' guidelines (2001), the precision of the model constructed for the remaining parameters ($0.51 \leq r^2 \leq 0.65$) may be considered acceptable for screening purposes, enabling samples to be classified as having high, medium or low values. This was achieved using a rapid, non-destructive handheld sensor in intact mandarins, thus providing the growers and the citrus industry with an instant response and enabling mandarin harvesting to be started at the optimum time.

Whilst no research published to date on NIR applications in intact mandarins has examined the correlation between spectroscopic data and external quality parameters, a number of studies have addressed the use of NIR spectroscopy for predicting external parameters such as fruit weight and equatorial diameter. Pérez-Marín et al. (2009), using the same MEMS-based device used here, reported similar results for the prediction of weight (RPD = 1.40; CV = 17.30%), but slightly poorer results for equatorial diameter (RPD = 1.31; CV = 9.83%) in intact nectarines analyzed on-tree and during postharvest storage. Sánchez et al. (2011) improved on these results, obtaining RPD values of 1.45 and 1.44, for weight and equatorial diameter, respectively, i.e. similar to those obtained here.

Cayuela and Weiland (2010) used two NIRS spectrophotometers in reflectance mode – a diode array device (Labspec VIS/NIR, 500-2,300 nm) and an acousto-optic tunable filter (AOTF) instrument (Luminar 5030, 1,100-2,300 nm) – to predict the weight of intact oranges in the laboratory, recording RPD values of 3.91 and 3.43 and CV values of 15.41% and 17.58%, respectively; their results are thus better than those obtained here. However, as indicated earlier, this was a laboratory study and used instruments that differed in terms of both operating principle and spectral range from that used here.

The results obtained by applying the best calibration models to the external validation set are shown in Table 4.

Using the monitoring procedure outlined by Shenk et al. (2001) the prediction-statistic values obtained for most parameters, except a*, h* and color index, fell short of the limit recommended for routine application ($r^2 > 0.60$). However it should be stressed that SEP(c) and slope values were close to confidence limits and the bias was below confidence limits, suggesting that these NIRS equations for external parameters can be seen as a useful preliminary trial for the construction of accurate on-tree predictions for intact mandarins using a handheld MEMS spectrophotometer.

It should also be noted that for the parameters L*, b* and h*, external validation did not include all the samples in the validation set, since those displaying T statistic values greater than 2.5 were removed during cross-validation.

Specifically in the case of L*, four samples were removed; two displayed values (52.06 and 55.83) below the range finally used for the calibration set (58.12-71.74, Table 3), while the other two, though presenting in-range values (59.29 and 62.18), were under-represented in the set.

The same four samples were removed for b*; three displaying values (43.55 and 53.59) below the lower limit of the calibration range (53.78; Table 3), and the fourth presenting a value of 57.42.

Finally, samples with negative values for h* (N = 2; Fig. 1) were removed during external validation, since they lay outside the calibration-set range for this parameter (51.74-84.01; Table 3). Four further samples were removed because they displayed extreme values (85.38-88.63); as a result, these values were under-represented in the final calibration set. It should also be noted that three of these four samples were also removed during external validation for either L* or b*.

These results highlight the importance not only of ensuring a sufficient number of samples in the calibration set, but also of guaranteeing the adequate distribution and structure of the sample set.

3.4. Calibration for predicting internal quality parameters in mandarins

The calibration statistics for the prediction of physical properties (firmness, maximum penetration force, pericarp thickness, juice weight and juice content) and chemical parameters (soluble solids content, pH, titratable acidity and maturity index) in intact mandarins are shown in Table 5.

The best equations for predicting physical-chemical parameters were obtained using SNV + DT as scatter-correction treatments, though not in the case of SSC; this underlines the importance of selecting spectral-signal pretreatments as a function of the parameter to be analyzed (Delwiche and Reeves, 2004).

The models displaying the greatest predictive capacity were constructed using the second derivative of the spectrum, except for the parameters firmness, juice weight and SSC, where the first derivative provided better results.

With regard to texture-related parameters, models obtained for maximum penetration force displayed greater predictive capacity than those constructed for firmness (Table 5). The poor predictive capacity of firmness models highlights the difficulty in correlating destructive measurements made to a puncturing depth of 10 mm with non-destructive NIR measurements. As Peirs et al. (2002) have noted, NIR light will only penetrate usefully down to a depth of between 1 and 5 mm, depending on the wavelength, the instrument and the fruit ripeness stage. For maximum penetration force, the value of the coefficient of determination (r^2) (0.47) for the best model obtained suggests that this model would only enable discrimination between high and low maximum penetration force (Williams 2001).

These findings agree with those reported by Pérez-Marín et al., (2010), who stress the difficulty in predicting texture-related parameters (firmness and maximum penetration force) in certain fruits using NIRS technology with MPLS regression.

No references have been found in the literature to the measurement of these parameters in intact mandarins using NIRS technology. Hernández Gómez et al. (2006) measured compression force (i.e. the force required to compress a fruit by 3% of its diameter) in intact Satsuma mandarins, using a monochromator instrument with a spectral range of 350–2500 nm, obtaining a reasonable-to-good prediction performance (r^2 = 0.75; RPD = 1.77). However, this texture parameter was not measured in the present study, so findings cannot be compared.

The predictive capacity of the best model for predicting pericarp thickness may be considered acceptable for screening purposes, since the value recorded for the coefficient of determination for cross-validation (r^2 = 0.52) would enable samples to be classified as high, medium or low; given the link between pericarp thickness and fruit yield, this classification would be of considerable value to the citrus-fruit sector.

By contrast, the models obtained for predicting juice weight and juice content, both of which are of great importance to the industry, must be deemed unacceptable in view of the results obtained both at calibration (Table 5) and at external validation (Fig. 2).

Results for chemical parameters in intact mandarins are shown in Table 5. The predictive capacity of models for pH and maturity index may be considered acceptable (r^2 = 0.75 and 0.74; SECV = 0.09 and 1.16), whilst in terms of the recommendations made by Williams (2001), the precision of models for predicting soluble solids content and titratable acidity discriminated between high, medium and low values, with r^2 values of 0.55 and 0.64 for SSC and TA, respectively.

The predictive capacity of the SSC model was poorer than that reported for intact mandarins by Hernández-Gómez et al. (2006) (RPD = 4.00; CV = 1.67%) and by Liu et al. (2010) (RPD = 2.90; CV = 4.17%), although these authors used a monochromator in the 350-2500 nm range and a diode-array instrument in the 600-980 nm range, respectively. Values of the statistics for the models constructed in this study were better than those reported by Hernández-Goméz et al. (2006) for pH (RPD = 1.73; CV = 3.25%) and by Liu et al. (2010) for TA (RPD = 0.8; CV = 22.72%).

No published studies have addressed the calculation of the SSC:TA ratio using NIRS technology in intact mandarins.

Validation statistics for the prediction of internal physical-chemical quality parameters in intact mandarins both on-tree and at harvest are shown in Figure 2.

The model constructed for predicting pH in intact mandarins using a portable MEMS-NIR instrument met the validation requirements recommended by Shenk et al. (2001) in terms of r^2 and slope (r^2 > 0.6; slope > 0.90) and both the SEP(c) and the bias were within confidence limits: the equation thus ensures accurate prediction, and can be

applied routinely. For pericarp thickness, maximum penetration force and the other internal chemical quality parameters studied, it should be stressed that SEP(c) and bias lay within confidence limits, although r^2 and slope results did not always attain recommended minimum values, indicating that the NIRS equations constructed here can be considered as a first step in the fine-tuning of NIRS technology for the on-tree monitoring of internal quality parameters in mandarins.

The models predicted firmness, juice weight and juice content in validation-set samples with low values for r^2 (0.15, 0.28 and 0.22, respectively), in neither case meeting the recommendations of Shenk et al. (2001). These models are thus not suitable for routine applications.

Comparison of the data in Table 2 and Figure 2 shows that not all samples in the initial external validation set were used for the prediction of juice content, SSC, TA, or maturity index. During external validation of models for predicting juice content, two samples were removed because they displayed values of 11.12% and 19.18%, both well below the lower limit of the range finally used for the calibration set (21.99-55.89%, Table 5). In the external validation for SSC, one sample with a value of 15.40 % was removed because, though lying within the range of the final calibration set (9.95-15.65; Table 5), it was under-represented in that set due to the earlier removal – during the calibration stage – of four samples, three of which presented values of between 14.60 and 15.45%; as a result, the representativity of this interval was reduced to a single sample with an SSC value of over 15%.

In the external validation for titratable acidity and maturity index, a total of four samples were removed (the same four for each parameter), presenting TA values of between 1.51 and 2.06% citric acid, and maturity index values of between 6.14 and 8.65. Again, removal of samples in the calibration set (N = 6 for TA; N = 8 for maturity

index) meant that samples were found to be out-of-range in the validation set or very under-represented in the final calibration set.

4. Conclusions

NIRS technology using the latest-generation, hand-held compact MEMS instrument proved suitable for assessing a large number of internal and external quality parameters in intact mandarins during on-tree ripening, allowing ripeness to be evaluated not only in terms of visual appearance but also in terms of taste and texture. This could lead to major changes in harvesting techniques, by providing farmers with a precise and accurate indication of the fruit's internal and external quality, thus enabling selective harvesting. To our knowledge, this is the first attempt to use NIR spectroscopy for the on-tree monitoring of ripening in mandarins. Over the coming years, however, recalibrations may be required in order to enhance the robustness of the models obtained; there is also a need for databases whose structure and distribution fully represent the spectral variability likely to appear during on-tree analysis.

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References

Antonucci, F., Pallottino, F., Paglia, G., Palma, A., D'Aquino, S., Menesatti, P., 2011. Non-destructive estimation of mandarin maturity status through portable VIS-NIRS spectrophotometer. Food and Bioprocess Technology 4, 809–813.

Arias, C.J., Toledo, J., 2000. Manual de Manejo Postcosecha de Frutas Tropicales (papaya, piña, plátano y cítricos). Food and Agriculture Organization of the United Nations, Publishing and Multimedia Service, Rome, Italy.

Barnes, R.J., Dhanoa, M.S., Lister, S.J., 1989. Standard Normal Variate Transformation and De-trending of near infrared diffuse reflectance spectra. Applied Spectroscopy 43, 772–777.

Cayuela, J.A., Weiland, C., 2010. Intact orange quality prediction with two portable NIR spectrometers. Postharvest Biology and Technology 58, 113–120.

Delwiche, S.R., Reeves, J.B., 2004. The effect of spectral pretreatments on the PLS modeling of agricultural products. Journal of Near Infrared Spectroscopy 12, 177–182.

Fernández-Cabanás, V.M., Garrido-Varo, A., Pérez-Marín, D., Dardenne, P., 2006. Evaluation of pretreatment strategies for near infrared spectroscopy calibration development of unground and ground compound feedingstuffs. Applied Spectroscopy 60, 17–23.

García-Luis, A., Oliveira, M.E., Bordón, Y., Siquiera, D.L., Tominaga, S., Guardiola, J.L., 2002. Dry matter accumulation in citrus fruit is not limited by transport capacity of the pedicel. Annals of Botany 90, 755–764.

Geller, Y., 2007. Using MEMS technology for cost effective recycling of plastics. MOEMS and Miniaturized Systems VI. In: Dickensheets, D.L., Gogoi, B.P., Schenk, H. (Eds.), Proceedings of the SPIE, Volume 6466, pp. 646604–1–646604–7.

González-Caballero, V., Sánchez, M.T., Fernández-Novales, J., López, M.I., Pérez-Marín, D., 2012. On-vine monitoring of grape ripening using Near-Infrared Spectroscopy. Food Analytical Methods DOI: 10.1007/s12161-012-9389-3.

Greensill, C.V., Walsh, K., 2002. Calibration transfer between miniature photodiode array-based spectrometers in the near infrared assessment of mandarin soluble solids content. Journal of Near Infrared Spectroscopy 7, 27–35.

Guthrie, J.A., Walsh, K.B., Reid, D.J., Lienberg, C.J., 2005a. Assessment of internal quality attributes of mandarin fruit I. NIR calibration model development. Australian Journal of Agricultural Research 56, 405–416.

Guthrie, J.A., Reid, D.J., Walsh, K.B., 2005b. Assessment of internal quality attributes of mandarin fruit II. NIR calibration model robustness. Australian Journal of Agricultural Research 56, 417–426.

Hernández-Gómez, A.H., He, Y., Pereira, A.G., 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.

ISI, 2000. The Complete Software Solution Using a Single Screen for Routine Analysis, Robust Calibrations and Networking. Manual, FOSS NIRSystems/Tecator. Infrasoft International, Silver Spring, MD.

Kawano, S., Fujiwara, T., Iwamoto, M., 1993. Nondestructive determination of sugar content in Satsuma mandarin using near infrared (NIR) transmittance. Journal of the Japanese Society for Horticultural Science 62, 465–470.

Liu, Y., Sun, X., Zhang, H., Aiuguo, O., 2010. Nondestructive measurement of internal quality of Nanfeng mandarin fruit by charge coupled device near infrared spectroscopy. Computers and Electronics in Agriculture 715, S10–S14.

McGlone, V.A., Fraser, D.G., Jordan, R.B., Künnemeyer, R., 2003. Internal quality assessment of mandarin fruit by vis/NIR spectroscopy. Journal of Near Infrared Spectroscopy 11, 323–332.

Miyamoto, J., Kitano, Y., 1995. Non-destructive determination of sugar content in Satsuma mandarin fruit by near infrared transmittance spectroscopy. Journal of Near Infrared Spectroscopy 3, 227–237.

Miyamoto, K., Kawauchi, M., Fukuda, T., 1998. Classification of high acid fruits by partial least squares using the near infrared transmittance spectra of intact Satsuma mandarins. Journal of Near Infrared Spectroscopy 6, 267–271.

Naes, T., Isaksson, T., Fearn, T., Davies, A., 2002. A User-Friendly Guide to Multivariate Calibration and Classification. NIR Publications, Chichester, UK.

Obenland, D., Collin, S., Sievert, J., Fjeld, K., Doctor, J., Arpaia, M.L., 2008. Commercial packing and storage of navel oranges alters aroma volatiles and reduces flavor quality. Postharvest Biology and Technology 47, 159–167.

Peirs, A., Scheerlinck, N., Touchant, K., Nicolaï, B.M., 2002. Comparison of Fourier transform and dispersive near-infrared reflectance spectroscopy for apple quality measurements. Biosystems Engineering 81, 305–311.

Pérez-Marín, D., Garrido-Varo, A., Guerrero, J.E., 2005. Implementation of LOCAL algorithm with near-infrared spectroscopy for compliance assurance in compound feedingstuffs. Applied Spectroscopy 59, 69–77.

Pérez-Marín, D., Sánchez, M.T., Paz, P., Soriano, M.A., Guerrero, J.E., Garrido-Varo, A., 2009. Non-destructive determination of quality parameters in nectarines during on-tree ripening and postharvest storage. Postharvest Biology and Technology 52, 180–188.

Pérez-Marín, D., Paz, P., Guerrero, J.E., Garrido-Varo, A., Sánchez, M.T., 2010. Miniature handheld NIR sensor for the on-site non-destructive assessment of post-harvest quality and refrigerated storage behavior in plums. Journal of Food Engineering 99, 294–302.

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. LWT – Food Science and Technology 44, 1405–1414.

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, 130–135.

Sánchez, M.T., De la Haba, M.J., Benítez-López, M., Fernández-Novales, J., Garrido-Varo, A., Pérez-Marín, D., 2012. Non-destructive characterization and quality control of intact strawberries based on NIR spectral data. Journal of Food Engineering 110, 102–108.

Shenk, J.S., Westerhaus, M.O., 1991a. Population definition sample selection and calibration procedures for near infrared spectra and modified partial least squares regression. Crop Science 31, 469–474.

Shenk, J.S., Westerhaus, M.O., 1991b. Population structuring of near infrared spectra and modified partial least squares regression. Crop Science 31, 1548–1555.

Shenk, J.S., Westerhaus, M.O., 1995a. Analysis of Agriculture and Food Products by Near Infrared Reflectance Spectroscopy. Monograph. NIRSystems Inc., Silver Spring, MD.

Shenk, J.S., Westerhaus, M.O., 1995b. Routine Operation, Calibration, Development and Network System Management Manual. NIRSystems Inc., Silver Spring, MD.

Shenk, J.S., Workman, Jr. J.J., Westerhaus, M.O., 2001. Application of NIR spectroscopy to agricultural products. In: Burns, D.A., Ciurczak, E.W. (Eds.),

Handbook of Near Infrared Analysis, Practical Spectroscopy Series, vol. 27, second ed. Marcel Dekker, New York, pp. 419–473.

Tsuchikawa, S., Sakai, E., Inoue, K., Miyamoto, K., 2003. Application of time-of-flight near-infrared spectroscopy to detect sugar and acid content in Satsuma mandarin. Journal of the American Society for Horticultural Science 28, 391–396.

Walsh, K.B., Golic, M., Greensill, C.V., 2004. Sorting of fruit using near infrared spectroscopy: application to a range of fruit and vegetables for soluble solids and dry matter content. Journal of Near Infrared Spectroscopy 12, 141–148.

Williams, P.C., 2001. Implementation of near-infrared technology. In: Williams, P.C., Norris, K.H. (Eds.), Near-Infrared Technology in the Agricultural and Food Industries. AACC, Inc., St. Paul, Minnesota, pp. 145–171.

Xudong, S., Hailiang, Z., Yande, L., 2009. Nondestructive assessment of quality of Nanfeng mandarin fruit by a portable near infrared spectroscopy. International Journal of Agricultural and Biological Engineering 2, 65–71.

Zude, M., Pflanz, M., Kaprielian, C., Aivazian, B.L., 2008. NIRS as a tool for precision horticulture in the citrus industry. Biosystems Engineering 99, 455–459.

Table 1

Changes in external and internal quality parameters of mandarins during on-tree ripening as a function of harvest date.

Constituent	October 29	November 05	November 12	November 19	December 03	December 16
Weight (g)	101.60 ± 26.73$^{(a)}$	115.36 ± 26.73$^{(a)\,(b)}$	121.10 ± 32.22$^{(b)}$	126.93 ± 34.43$^{(b)\,(c)}$	140.36 ± 33.09$^{(c)\,(d)}$	146.35 ± 34.04$^{(d)}$
Equatorial diameter (mm)	50.95 ± 4.71$^{(a)}$	62.93 ± 6.08$^{(b)}$	63.79 ± 6.00$^{(b)}$	65.16 ± 6.77$^{(b)\,(c)}$	68.17 ± 6.27$^{(c)\,(d)}$	68.20 ± 5.88$^{(d)}$
Axial diameter (mm)	59.50 ± 5.51$^{(c)}$	52.64 ± 3.98$^{(a)}$	53.41 ± 5.07$^{(a)}$	53.00 ± 4.92$^{(a)}$	56.84 ± 3.93$^{(b)}$	56.36 ± 5.39$^{(b)}$
L*	58.09 ± 6.30$^{(a)}$	64.90 ± 4.15$^{(c)}$	64.55 ± 3.43$^{(c)}$	64.88 ± 1.80$^{(c)}$	62.52 ± 1.93$^{(b)}$	61.86 ± 2.32$^{(b)}$
a*	-2.63 ± 9.80$^{(a)}$	12.77 ± 11.62$^{(b)}$	22.22 ± 8.15$^{(c)}$	27.74 ± 8.85$^{(d)}$	35.03 ± 3.54$^{(e)}$	37.53 ± 2.76$^{(e)}$
b*	52.11 ± 9.82$^{(a)}$	62.25 ± 5.82$^{(c)\,(d)}$	63.96 ± 4.46$^{(d)}$	63.37 ± 2.46$^{(d)}$	60.66 ± 2.81$^{(b)\,(c)}$	59.86 ± 3.22$^{(b)}$
C*	53.12 ± 9.42$^{(a)}$	64.46 ± 6.88$^{(b)}$	68.09 ± 5.76$^{(c)}$	69.70 ± 3.18$^{(c)\,(d)}$	70.15 ± 2.38$^{(c)\,(d)}$	70.75 ± 1.89$^{(d)}$
h*	-17.06 ± 80.14$^{(a)}$	60.55 ± 51.10$^{(b)}$	71.17 ± 6.23$^{(b)}$	66.61 ± 7.31$^{(b)}$	60.00 ± 3.17$^{(b)}$	57.86 ± 3.09$^{(b)}$
Color index	-1.80 ± 3.68$^{(a)}$	3.00 ± 2.98$^{(b)}$	5.34 ± 1.86$^{(c)}$	6.80 ± 2.33$^{(d)}$	9.30 ± 1.37$^{(e)}$	10.25 ± 1.58$^{(e)}$
Firmness (N)	13.77 ± 8.30$^{(c)}$	11.30 ± 7.44$^{(b)\,(c)}$	12.58 ± 7.13$^{(c)}$	8.84 ± 3.50$^{(a)\,(b)}$	7.37 ± 3.42$^{(a)}$	6.78 ± 2.53$^{(a)}$
Maximum penetration force (N)	52.57 ± 11.48$^{(d)}$	40.65 ± 8.52$^{(c)}$	42.97 ± 9.21$^{(c)}$	35.88 ± 7.75$^{(b)}$	30.07 ± 6.04$^{(a)}$	27.78 ± 4.56$^{(a)}$
Pericarp thickness (mm)	3.77 ± 0.84$^{(a)\,(b)}$	3.41 ± 0.69$^{(a)}$	3.50 ± 0.78$^{(a)}$	3.54 ± 0.68$^{(a)}$	4.09 ± 0.70$^{(b)}$	3.58 ± 0.75$^{(a)}$
Juice weight (g)	31.26 ± 15.20$^{(a)}$	44.37 ± 12.02$^{(b)}$	45.06 ± 19.06$^{(b)}$	48.53 ± 15.82$^{(b)\,(c)}$	52.02 ± 15.08$^{(b)\,(c)}$	53.20 ± 17.94$^{(c)}$
Juice content (%)	29.52 ± 9.80$^{(a)}$	37.41 ± 6.15$^{(b)}$	35.98 ± 8.58$^{(b)}$	38.07 ± 5.87$^{(b)}$	36.91 ± 6.21$^{(b)}$	35.89 ± 8.01$^{(b)}$
Soluble solids content (%)	12.05 ± 1.21$^{(a)}$	12.08 ± 1.18$^{(a)}$	12.65 ± 1.13$^{(a)\,(b)}$	12.76 ± 1.27$^{(b)}$	12.65 ± 0.86$^{(a)\,(b)}$	12.81 ± 1.13$^{(b)}$
pH	3.20 ± 0.12$^{(b)}$	3.03 ± 0.11$^{(a)}$	3.13 ± 0.10$^{(b)}$	3.19 ± 0.12$^{(b)}$	3.29 ± 0.13$^{(c)}$	3.41 ± 0.16$^{(d)}$
Titratable acidity (% citric acid)	1.33 ± 0.17$^{(b)\,(c)}$	1.35 ± 0.21$^{(c)}$	1.33 ± 0.22$^{(b)\,(c)}$	1.33 ± 0.22$^{(b)(c)}$	1.22 ± 0.31$^{(b)}$	1.00 ±0 .17$^{(a)}$
Maturity index	8.86 ± 1.00$^{(a)}$	9.04 ± 1.35$^{(a)}$	9.68 ± 1.53$^{(a)}$	9.73 ± 1.26$^{(a)}$	10.86 ± 2.14$^{(b)}$	13.08 ± 1.66$^{(c)}$

Means in the same row bearing different superscripts differ significantly ($p < 0.05$).

Table 2

Range, mean, standard deviation (SD) and coefficient of variation (CV) for the parameters studied in calibration and validation sets.

Constituent	Set	N	Range	Mean	SD	CV (%)
Weight (g)	Calibration	140	54.91-221.50	130.05	36.14	27.79
	Validation	71	72.97-209.31	126.40	32.89	26.02
Equatorial diameter (mm)	Calibration	140	41.37-83.05	63.97	8.41	13.15
	Validation	71	42.65-80.21	62.28	7.42	11.91
Axial diameter (mm)	Calibration	140	42.10-72.18	55.55	5.63	10.14
	Validation	71	44.08-68.10	55.00	4.87	8.85
L*	Calibration	140	46.12-71.74	62.54	4.37	6.99
	Validation	71	52.06-67.34	62.99	3.42	5.43
a*	Calibration	140	-15.43-42.41	24.57	16.40	66.75
	Validation	71	-9.16-41.35	25.65	13.79	53.76
b*	Calibration	140	34.89-71.93	60.07	6.75	11.24
	Validation	71	43.55-71.43	60.98	4.72	7.74
C*	Calibration	140	37.61-75.28	66.74	8.43	12.63
	Validation	71	44.50-74.61	67.44	6.23	9.24
h*	Calibration	140	-89.88-89.70	46.47	49.24	105.96
	Validation	71	-80.70-88.67	63.03	26.59	42.19
Color index	Calibration	140	-8.73-13.56	6.15	4.99	81.14
	Validation	71	-4.04-13.18	6.67	3.92	58.77
Firmness (N)	Calibration	140	2.07-35.47	9.80	6.30	64.29
	Validation	71	2.80-29.72	8.92	5.17	57.96
Maximum penetration force (N)	Calibration	140	16.20-78.92	36.57	11.74	32.10
	Validation	71	21.69-67.16	35.68	9.79	27.44
Pericarp thickness (mm)	Calibration	140	1.64-5.65	3.61	0.76	21.05
	Validation	71	2.20-5.60	3.66	0.76	20.77
Juice weight (g)	Calibration	139	2.62-90.69	48.10	17.92	37.26
	Validation	71	10.01-87.81	45.21	17.25	38.16
Juice content (%)	Calibration	139	4.77-55.89	36.09	8.18	22.67
	Validation	71	11.12-49.23	35.11	7.68	21.87
Soluble solids content (%)	Calibration	137	9.95-15.65	12.58	1.18	9.38
	Validation	71	10.40-15.40	12.59	1.12	8.90
pH	Calibration	133	2.93-3.80	3.25	0.18	5.54
	Validation	70	2.87-3.67	3.24	0.18	5.56
Titratable acidity (% citric acid)	Calibration	124	0.71-2.08	1.19	0.26	21.85
	Validation	64	0.74-2.06	1.23	0.27	21.95
Maturity index	Calibration	124	5.41-17.37	11.01	2.36	21.44
	Validation	64	6.14-15.43	10.70	2.23	20.84

Table 3

Calibration statistics for weight, equatorial and axial diameters, and color parameters using the MPLS regression method.

Parameter	Mathematic treatment	Number	Range	Mean	SD	SEC	R^2	SECV	r^2	RPD	CV (%)
Weight (g)	1,5,5,1-SNV+DT	135	61.87-221.50	131.22	34.72	23.91	0.53	24.31	0.51	1.43	18.52
Equatorial diameter (mm)	1,5,5,1-None	137	41.37-83.05	64.23	8.23	5.33	0.58	5.63	0.54	1.46	8.76
Axial diameter (mm)	1,5,5,1-SNV+DT	128	42.10-66.51	55.56	5.11	2.45	0.77	3.21	0.61	1.59	5.78
L*	1,5,5,1-SNV′+DT	117	58.12-71.74	63.65	2.85	1.73	0.63	1.80	0.61	1.58	2.83
a*	1,10,5,1-None	133	-15.43-42.41	24.58	16.69	7.34	0.81	8.18	0.76	2.04	33.30
b*	1,5,5,1-SNV+DT	118	53.78-71.93	62.07	3.74	2.54	0.54	2.62	0.52	1.43	4.22
C*	1,5,5,1-SNV+DT	128	37.61-74.54	66.64	8.28	3.98	0.77	4.92	0.65	1.68	7.38
h*	2,10,5,1-None	111	51.74-84.01	63.29	7.46	4.56	0.63	5.71	0.52	1.31	9.03
Color index	1,10,5,1-None	136	-8.73-13.56	6.16	5.04	2.30	0.79	2.62	0.74	1.92	42.51

Table 4

Validation statistics for predicting external quality parameters in intact mandarins.

Parameter	Validation statistics					Control limits	
	SEP	SEP(c)	Bias	r^2	Slope	SEP(c)	Bias
Weight (g)	26.12	25.75	-5.32	0.39*	0.91	31.08	±14.35
Equatorial diameter (mm)	5.83	5.83	-0.71	0.39*	0.88*	6.93	±3.20
Axial diameter (mm)	4.36	4.39*	0.19	0.31*	0.57*	3.19	±1.47
L*	2.26	2.25	-0.33	0.47*	1.11	2.25	±1.04
a*	8.41	8.41	1.03	0.65	0.86*	9.54	±4.40
b*	3.03	3.02	-0.45	0.42*	1.07	3.30	±1.52
C*	5.92	5.83*	1.27	0.35*	0.56*	5.17	±2.39
h*	6.55	6.42*	1.50	0.64	1.43	5.93	±2.74
Color index	2.33	2.32	0.37	0.68	0.82*	2.99	±1.38

*Values exceeding control limits described in Materials and Methods Section.

Table 5

MPLS regression statistics for NIR-based models for predicting internal physical and chemical quality parameters in intact mandarins.

Parameter	Mathematic treatment	Number	Range	Mean	SD	SEC	R^2	SECV	r^2	RPD	CV (%)
Firmness (N)	1,5,5,1-SNV+DT	126	2.07-19.75	8.17	3.34	2.84	0.28	2.96	0.22	1.13	36.26
Maximum penetration force (N)	2,10,5,1-SNV+DT	136	16.20-75.36	36.02	11.01	7.80	0.50	8.07	0.47	1.36	22.40
Pericarp thickness (mm)	2,5,5,1-SNV+DT	139	1.64-5.65	3.61	0.76	0.51	0.55	0.53	0.52	1.43	14.79
Juice weight (g)	1,5,5,1-SNV+DT	135	6.19-9.69	48.10	16.90	13.81	0.33	14.15	0.30	1.19	29.41
Juice content (%)	2,5,5,1-SNV+DT	131	21.95-55.89	37.20	6.23	5.29	0.28	5.45	0.24	1.14	14.65
Soluble solids content (%)	1,5,5,1-None	133	9.95-15.65	12.54	1.13	0.68	0.64	0.76	0.55	1.49	6.06
pH	2,5,5,1-SNV+DT	128	2.83-3.80	3.25	0.18	0.08	0.79	0.09	0.75	1.97	2.76
Titratable acidity (% citric acid)	2,5,5,1-SNV+DT	118	0.71-1.75	1.17	0.23	0.14	0.67	0.14	0.64	1.68	11.93
Maturity index	2,10,5,1-SNV+DT	116	6.60-15.94	10.98	2.26	1.11	0.76	1.16	0.74	1.95	10.56

Fig. 1. Distribution of samples for color-related parameters (a*, h* and color index) and firmness for the calibration (N = 140) and validation (N = 71) sets.

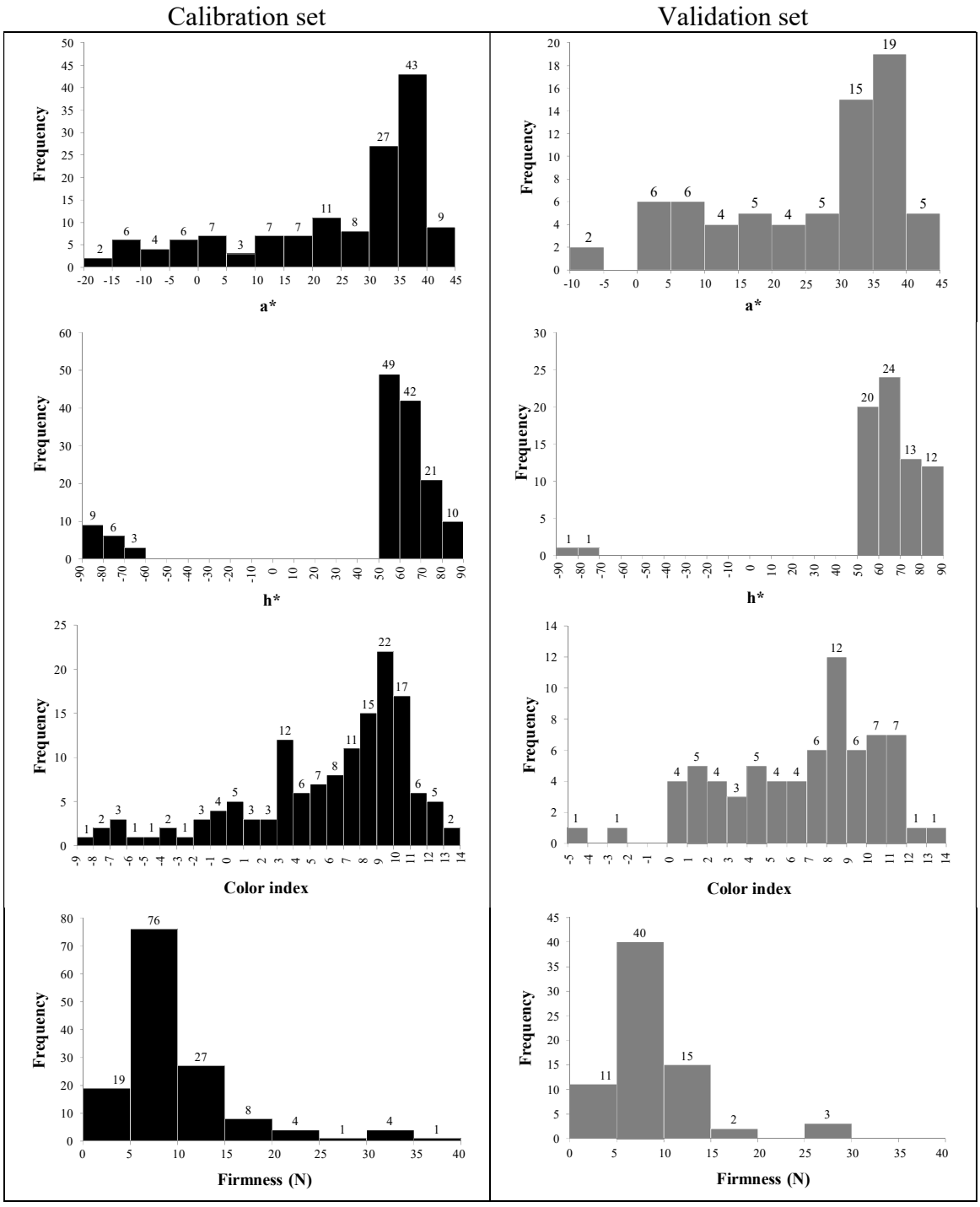
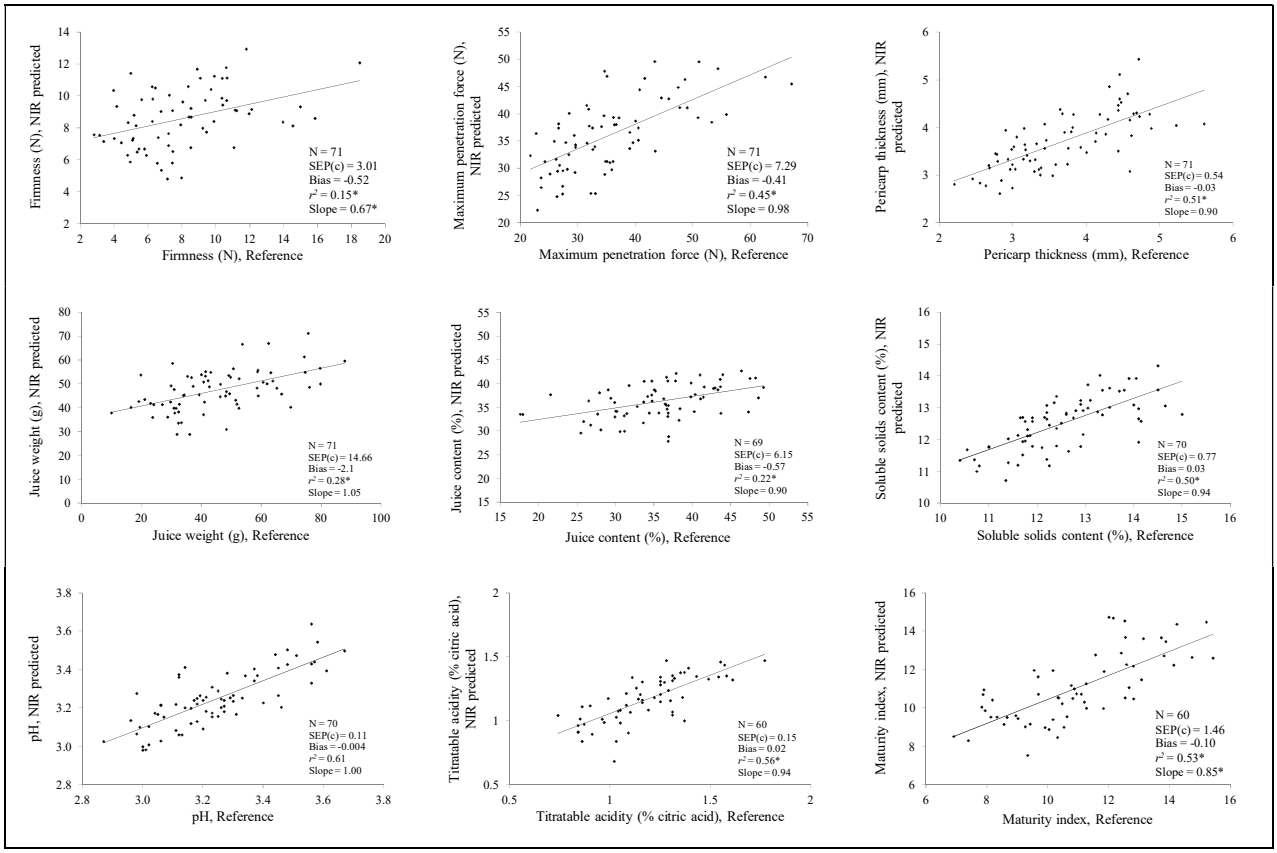


Fig. 2. Reference *versus* NIR-predicted data for the validation set.



*Values exceeding control limits described in Materials and Methods Section.