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# Non-invasive spectroscopic methods to estimate orange firmness, peel thickness, and total pectin content

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ABSTRACT

Orange firmness, peel thickness, and total pectin content are associated with fruit quality and denote important parameters for the food industry. These attributes are usually determined through destructive methods that can be time-consuming and also unable to monitor fruit quality over time. Therefore, non-invasive methods such time-domain nuclear magnetic resonance (TD-NMR), near-infrared (NIR), and mid-infrared (MIR) spectroscopies may represent efficient alternatives to evaluate these quality attributes. In this work, partial least square regression (PLSR) models of TD-NMR relaxometry as well as NIR and MIR spectroscopic data were used to predict firmness, peel thickness, and total pectin content of fresh Valencia oranges. Principal component analyses (PCA) were applied to explain the correlations of orange ripening stage, flowering, and crop season with its physicochemical parameters. Data obtained through standard destructive methods were used to calibrate and validate the PLSR models. NIR and MIR showed the best PLSR models for orange firmness, with Pearson correlation coefficients (r) of 0.92 and 0.84 and squared errors of prediction (SEP) equal to 6.22 and 9.05 N, respectively. Orange peel thickness PLSR model was validated only by TD-NMR ($r = 0.72$; SEP = 0.49 mm). TD-NMR and NIR also presented potential to predict total pectin orange in orange ($r = 0.76$ and 0.70; SEP = 5.76% and 5.04%, respectively). Therefore, NIR presented a higher potential to predict orange firmness than MIR and TD-NMR. On the other hand, TD-NMR showed a higher prediction power concerning peel thickness than NIR and MIR. Both NIR and TD-NMR methods showed similar prediction powers for total pectin content.



## 1. Introduction

Both for industry processing and for fresh market purposes, citrus fruits must be harvested taking into account their physiological maturity, since ripening is brought to an end when they are separated from the tree [1]. However, many physical and chemical changes may take place throughout fruit storage, leading to decreased fruit quality [2,3]. Fruit firmness has been used as a useful indicator of quality decay in fruits [4–6]. Therefore, numerous studies have been performed to find the postharvest effects on orange firmness [7,8]. Orange firmness is associated with its ripening stage, total pectin content, and peel thickness. Firmness has been determined by total pectin content, peel thickness, and compression [9], the latter implying fruit destruction.

Pectin is a polysaccharide composed of partially methyl esterified α1-4 D-galacturonic acid and that can be found in orange peel and juice. Pectins comprising more than 50% of methyl ester groups have been classified as high-methoxyl (HM) pectin, where a slow-methoxyl (LM) pectin are those having less than 50% of methyl ester groups. This classification may be used to determine pectin quality and suitable destination. In addition, pectin content may change during ripening and storage [10,11,12,13,14]. The standard pectin quantification method is a destructive, laborious, and time-consuming process that generates high volumes of chemical residues [15]. Therefore, a fast, simple, and non-invasive method to measure orange firmness, peel thickness, and total pectin content may represent an efficient alternative to evaluate these quality attributes.

Non-invasive methods, such as time-domain nuclear magnetic resonance (TD-NMR), near-(NIR), and mid-infrared (MIR) spectroscopies have been used to classify and determine quality parameters in intact fresh fruits and agri-food products [16–24]. NIR is a simple, low-cost method with potential for fresh fruit analysis, although it requires proper calibration and regression models. NIR has been used to quantify pectins and their constituents in Japanese pear [25] as well as firmness in mangoes [26]. MIR spectroscopy has been applied to determine sugar,

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organic acid, and polyphenol contents in apricot and apples [27,28]. NIR radiation has penetrates more than MIR and has been more suitable for analyses of both the bulk and intact samples [29]. However, the evaluation of orange soluble solid content using visible-shortwave near infrared (VIS–SWNIR) spectroscopy has been demonstrated that fruit peel can be a removed or use transmittance modes to obtain better results [30]. In addition, the prediction models for apple firmness based on NIR were significantly influenced by peel [31]. However, the peel interference should be taken into account in different ways due to the limitation related to light penetration [30]. Peel might also be removed to improve prediction effectiveness, but this would not enable a non-invasive approach as well. Hence, citrus peel thickness can be a challenge for infrared spectroscopic analyses due to its composition that significantly absorbs light. However, such absorption can be correlated with important quality attributes, including firmness, peel thickness, and total pectin content [30]. TD-NMR relaxometry based on $^1$H transverse relaxation time ($T_2$) can be applied to sample that have not been prepared and/or that present light diffusion hurdles, for which neither NIR nor MIR would be suitable. Thus, TD-NMR has been demonstrated as an interesting tool to classify oranges and plums in terms of their sensory attributes (*e.g.*, sweetness in fresh fruits) [16,32]. Changes in $T_2$ values could be associated with different water compartments on injured apples, indicating the potential of this technique for identifying internal fruit damage [33].

Given the above, the main goal of this work was to evaluate the prediction power of TD-NMR, NIR, and MIR techniques associated with multivariate analyses to estimate, in a non-invasively fashion, orange firmness, peel thickness, and total pectin content.

## 2. Materials and methods

### 2.1. Materials

Valencia oranges (450 units) were harvested at the beginning, middle, and end of the 2015 crop season at commercial farms located in São Paulo state, Brazil. In each harvest, fruits from the first and fourth flowering stages were collected.

### 2.2. Fruit characterization

Twenty fruits from each flowering stage and each harvest were characterized as to (1) peel color, in a Konica Minolta Sensing colorimeter (Tokyo, Japan) that expressed the results in L (lightness), a*, b*, hue angle ($\tan -1 = b^*/a^*$), and chromaticity $\sqrt{a^2 + b^2}$ values in the CIE Lab scale; (2) equatorial fruit diameter, which was measured with a manual caliper and expressed in millimeters; (3) total soluble solids (TSS), determined in the fresh juice with a refractometer (Atago Co, Brix-Meter, Tokyo) and expressed as °Bx; and (4) pH, which was measured in the PHS-3B pH meter in 20 ml of juice. All samples were analyzed in triplicate.

### 2.3. TD-NMR measurements

Intact oranges were analyzed in a SLK-MRI-1400 spectrometer (Spinlock Magnetic Resonance Solution, Cordoba, Argentina) equipped with a 0.23-T (9 MHz for $^1$H) Halbach permanent magnet and a 10-cm-diameter bore. Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence [34] was used for $^1$H transverse relaxation time measurement ($T_2$), with 90° and 180° pulses of 32 and 64 μs, echo time (τ) of 500 μs, 1500 echoes, 8 scans, and a recycle time of 5 s.

### 2.4. Infrared spectra acquisition

The NIR spectra were acquired in reflectance mode with a resolution of 16 $cm^{-1}$ in a Spectrum 100N spectrometer (Perkin-Elmer Corp, Norwalk, CT, USA). The MIR spectra were acquired in a Cary630 spectrometer (Agilent, Walnut Creek, CA, USA) equipped with an ATR accessory for acquiring data from 4000 to 700 $cm^{-1}$. In order to improve the signal-to-noise ratio, a resolution of 4 $cm^{-1}$ and 64 scans were used. The NIR and MIR measurements were performed at three spots within the equatorial region of the same fruit.

### 2.5. Reference analyses

#### 2.5.1. Fruit firmness

Orange firmness was measured using a texture analyzer (XT-Plus, Stable Microsystems). The analyses were performed using a 4-mm-diameter cylindrical probe, at a speed of 5 mm $s^2$, and a compression depth of 18 mm. The maximum force (N) was recorded and as attributed to fruit firmness. The measurements were performed at the same equatorial region that had been used in NIR and MIR measurements.

#### 2.5.2. Peel thickness

Peel thickness was measured at the aforementioned equatorial regions (*i.e.*, used in NIR and MIR measurements) of the sliced fruits, using a Mitutoyo manual caliper, model 500-197-30B, and expressed as millimeters [35].

#### 2.5.3. Total pectin content

The total pectin content was measured in accordance with the method proposed by McCready and McComb [15]. One gram of fresh orange peel previously crushed and homogenized with 25 ml of ethyl alcohol 95% was stored under refrigerator for 30 min. Then, the samples were filtered to remove soluble sugars. The filter residue was washed twice with approximately 10 ml of ethanol 75%, transferred to Erlenmeyer containing 50 ml of Versene solution, and having pH adjusted to 11.5. The samples were stored under refrigerator for another 30 min before the pH was adjusted to 5–5.05. Once pH was corrected, 100 mg of pectinase enzyme was added to the solution, which was stirred for 1 h. Finally, samples were filtered and the volume was adjusted to 100 ml with the Versene solution. The filtrate was used to quantify the galacturonic acid content using the *m*-hydroxydiphenyl method [36]. Results were expressed as percentage of total pectin.

**Table 1**
Physicochemical data expressed as mean values ± standard deviations of triplicate readings, according to orange flowering and crop season.

| | Harvest 1 | | Harvest 2 | | Harvest 3 | |
|---|---|---|---|---|---|---|
| Analysis/flowering | 1° | 4° | 1° | 4° | 1° | 4° |
| Diameter (mm) | 108.60 ± 34.10 | 63.55 ± 0.63 | 75.59 ± 0.66 | 70.49 ± 0.71 | 75.85 ± 0.81 | 64.95 ± 0.63 |
| L | 62.73 ± 0.71 | 52.32 ± 1.01 | 64.59 ± 0.50 | 50.07 ± 0.95 | 62.64 ± 1.19 | 53.43 ± 1.41 |
| Hue | 95.95 ± 0.84 | 115.47 ± 0.70 | 91.33 ± 0.69 | 116.58 ± 0.80 | 96.85 ± 1.29 | 114.33 ± 1.71 |
| Chroma | 58.72 ± 1.15 | 41.31 ± 0.90 | 59.79 ± 0.86 | 39.18 ± 1.01 | 53.43 ± 1.48 | 42.20 ± 1.57 |
| TSS (°Bx) | 9.13 ± 0.20 | 8.061 ± 0.07 | 9.36 ± 0.17 | 8.49 ± 0.13 | 8.28 ± 0.17 | 7.81 ± 0.15 |
| pH | 3.32 ± 0.02 | 2.84 ± 0.01 | 3.59 ± 0.02 | 3.23 ± 0.03 | 3.78 ± 003 | 3.18 ± 0.03 |

### 2.6. Data analysis

Origin8.1 (OriginLab, Northampton, MA, USA) and Pirouette v. 4.5 (Infometrix, Inc. Bothell, WA) softwares were used for data processing. The average of triplicates was considered. The independent data (X) matrix was composed of instrumental data individually acquired for each instrument (TD-NMR, MIR, and NIR). Also, the dependent (Y) data matrix was contained the reference analysis. Matrices X and Y were mean-centered.

Principal component analysis (PCA) was used to reduce the dimensionality of the X (TD-NMR, MIR, and NIR) data. In addition, another PCA analysis was also performed without instrumental data and using only diameter, L, Hue, Chroma, TSS, and pH values as variables to explain the correlations between orange flowering, and crop season, that were used as classes.

TD-NMR data were pre-processed through normalization by max (0–1) and then smothered by a second-order polynomial Savitzky-Golay algorithm with a 65-point window to improve the mathematical regression by reducing eventual noise. NIR data were pre-processed by means of a standard normal variation (SNV), where as MIR data were normalized by max (0–1) followed by second derivative of a second-order polynomial with a 35-point window.

Signal-free MIR spectra regions (*i.e.*, from 4000 to 3750 $cm^{-1}$ and between 2750 and 1780 $cm^{-1}$) were not used in the models. Also, the first ten variables and variables after 1 s of TD-NMR decay were excluded due to random instrumentation errors and noise, respectively.

The PLSR models for total pectin content considered 125 reference measurements and their respective spectral data. The PLSR model for firmness and peel thickness was built with standard and spectral data from 110 oranges. Calibration and external validation models were developed using 70% and 30% of the data, respectively. Calibration models were cross-validated by leave-one-out algorithm on the calibration set. Pearson correlation coefficient (r) and squared error of prediction (SEP) were used to evaluate the model performance on the prediction set (30% of the samples).

## 3. Results and discussion

### 3.1. PCA analysis

Table 1 shows the average values and standard deviations of physical and chemical data applied in triplicate (diameter, color, TSS content, and pH) of the studied oranges, according to the first and fourth flowerings and harvest time.

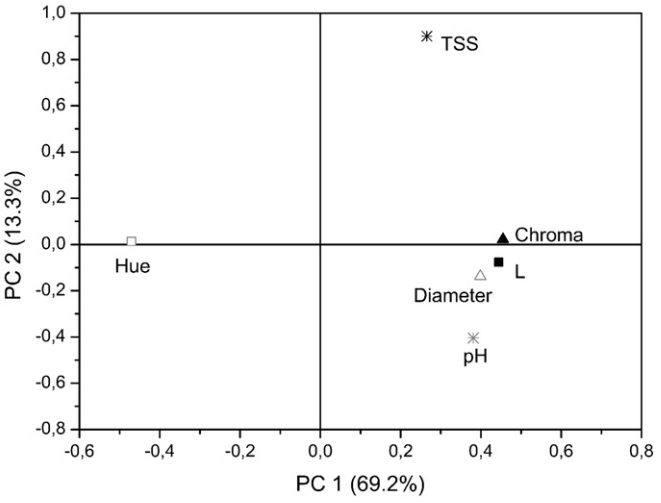


**Fig. 2.** Principal component analysis loadings of the physicochemical data of the first and fourth flowerings (Fig. 1).

Figs. 1 and 2 present the score and loading plots using principal components 1 (PC1) and 2 (PC2) for oranges harvested in the first and fourth flowerings, with regard to the physicochemical data. Fig. 1 shows a very good separation between the two flowering stages. The PCA of the first and fourth flowerings showed 69.2 and 13.3% of variance in PC1 and PC2, respectively. This discrimination was more influenced by PC1 due to the effects that Hue, diameter, and pH of flowerings played on PC weights, locating samples having low values on the left side of PC1 and those with high values on the right (Fig. 2).

On the other hand, no significant differences were observed among the studied harvest times (beginning, middle, and end of crop season) (Fig. 3). This agreed well with the results shown in Table 1, as similar ripening characteristics were obtained for all harvests, which in turn indicates a good variation distribution of harvest sampling.

Legend: 1°: first flowering; 4°: fourth flowering; L (lightness); Hue (hue angle); Chroma (chromaticity); TSS (total soluble solids, expressed as °Bx); pH (hydrogenionic potential); ± standard deviation.

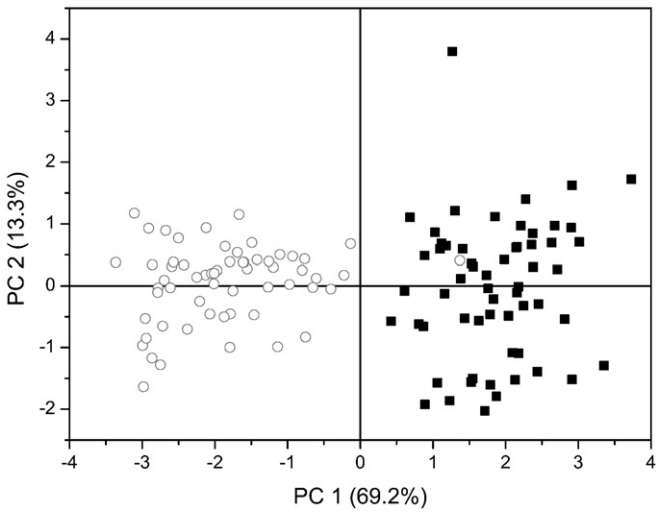


**Fig. 1.** Scores of principal components 1 (PC 1) and 2 (PC 2), as obtained through principal component analysis based upon the physicochemical data of the first (■) and fourth (○) flowerings.

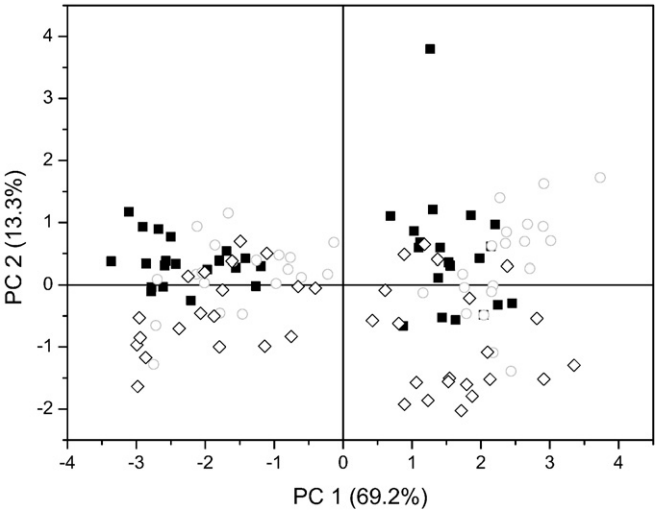


**Fig. 3.** Scores of principal components 1 (PC 1) and 2 (PC 2), as obtained through principal component analysis based upon the physicochemical data of harvests 1 (■), 2 (○), and 3 (◇).

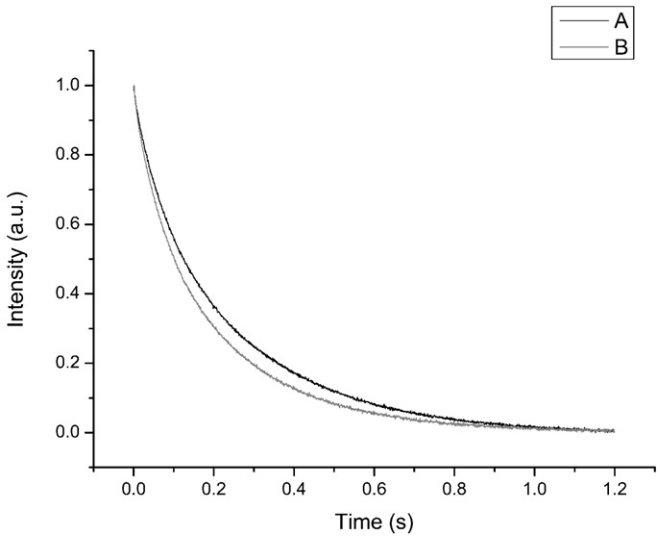


**Fig. 4.** Carr–Purcell–Meiboom–Gill (CPMG) decays of two oranges with different firmness, peel thickness, and total pectin content.

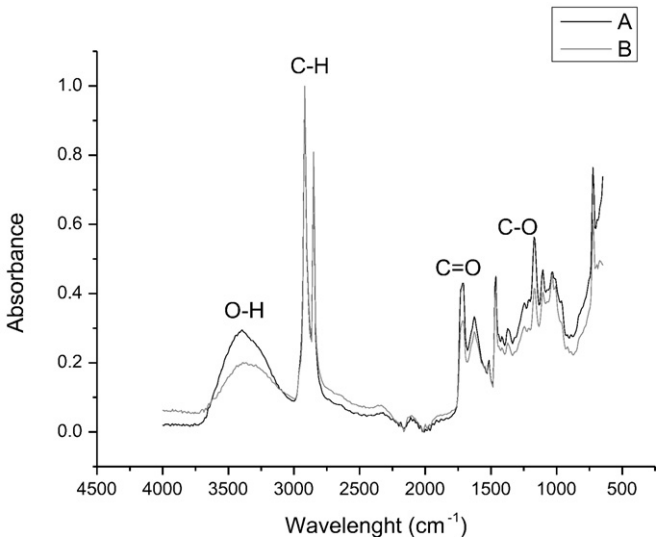


**Fig. 6.** Mid-infrared spectra of two orange fruits featuring different firmness.

### 3.2. Qualitative analyses using TD-NMR, NIR, and MIR spectra

Fig. 4 shows TD-NMR signals obtained with CPMG sequence of two oranges with peel thicknesses of 3.37 (sample A) and 5.73 mm (sample B). The same oranges revealed total pectin contents in peel equal to 32.62% and 44.14% as well as firmness of 35.20 (sample A) and 41.40 N (sample B), respectively.

Fig. 5 shows NIR spectra of the same samples addressed in Fig. 4. NIR spectrum A showed stronger O—H stretching peaks at 4545–5000 and 7143–6250 $cm^{-1}$,which have been associated with the presence of water within the fruit. The peaks at 9000 and 8000 $cm^{-1}$ have been attributed to C—H stretching of polysaccharides and essential oils that were present in orange peel.

Fig. 6 shows MIR spectra of the same orange fruits shown in Figs. 4 and 5. Such spectra showed the characteristic absorption of water O—H groups between 3500 and 3073 $cm^{-1}$. The strong peaks from 3000 to 2800 $cm^{-1}$ have been assigned to C—H bonds among organic compounds. The absorption peaks between 1790 and 1706 $cm^{-1}$ are related to C═O groups, whereas those at 1000–1200 $cm^{-1}$are attributed to C—O groups. These peaks have been associated with polysaccharides and essential oils [37].

Therefore, by analyzing Figs. 4–6, tone may observe that all three methods were shown to potentially detect orange firmness, peel thickness, and total pectin content in a non-invasively fashion. These potentials were quantitatively evaluated using PLSR regression models.

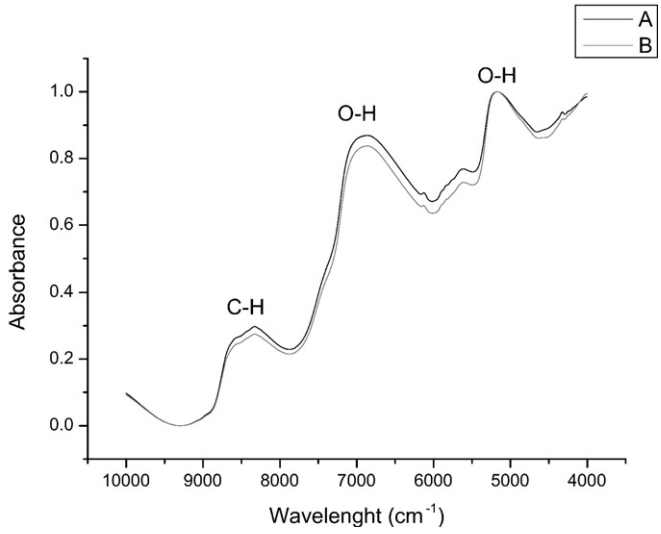


**Fig. 5.** Near-infrared spectra of two oranges with different firmness, peel thickness, and total pectin content.

### 3.3. TD-NMR PLSR models

TD-NMR data collected with CPMG pulse sequence are related to transverse relaxation time of water entrapped in oranges, which depends on their TSS content and on the pH of their juices. Table 2 present the calibration and internal validation PLSR models for firmness, peel thickness, and total pectin content using TD-NMR data. Both firmness and peel thickness calibration PLSR models were obtained using 734 variables of the original 1500. Firmness and peel thickness PLSR models showed the lowest values of squared error of validation (SEV; 8.86 N and 0.51 mm, respectively) and the highest rVal values (0.80 and 0.71, respectively). Total pectin content PLSR model obtained from TD-NMR data showed the best performance using 1323 variables. Total pectin content results are characterized by the lowest SEV and rVal values (5.13% and 0.76, respectively) in the internal validation (Table 2).

Table 3 demonstrates the external validation PLSR models as to firmness, peel thickness, and total pectin content using TD-NMR data. Such results evidenced the potential of TD-NMR data in predicting these three physical parameters of Valencia oranges. However, the best prediction power was obtained for peel thickness because of the high r and small SEP.

The correlation coefficients of validation model showed a small decay when compared to the calibration model (Table 2) concerning fruit firmness, peel thickness, and total pectin content. SEV and SEC values were similar for firmness and peel thickness, but SEV was fivefold increased for total pectin content when compared to SEC.

**Table 2**
Partial least squares regression for calibration models using time-domain nuclear magnetic resonance data.

| | SEV | rVal | SEC | rCal | N |
|---|---|---|---|---|---|
| Firmness (N) | 8.86 | 0.80 | 8.09 | 0.85 | 75 |
| Peel thickness (mm) | 0.51 | 0.71 | 0.45 | 0.78 | 75 |
| Total pectin content(%) | 5.13 | 0.76 | 4.72 | 0.82 | 78 |

SEV and SEC = squared errors of validation and calibration, respectively; n = sample universe; rVal and rCal = Pearson correlation coefficients of validation and calibration, respectively.

**Table 3**
External validation of the partial least squares regression models based upon time-domain nuclear magnetic resonance data.

| | SEP | r | n |
|---|---|---|---|
| Firmness (N) | 8.27 | 0.64 | 31 |
| Peel thickness (mm) | 0.49 | 0.72 | 31 |
| Total pectin content (%) | 5.76 | 0.76 | 37 |

SEP = squared error of prediction; r = Pearson correlation coefficient; n = sample universe for validation.

The thickness of Valencia orange peel of internal canopies was significantly higher than those of external fruits. This variation is a result of the mineral nutrition of the tree [38]. During fruit ripening, juice mass increases while peel thickness decreases. Therefore, there is a greater percentage of free water in orange. These results suggest that the percentage of free water in oranges of different flowerings and harvests denote an important variable to approximate the correlation between TD-NMR and fruit peel thickness, corroborating which has been discuss at the end of item 3.2.

Orange peel thickness variations as induced by fruit ripening stage or tree mineral nutrition were detected using TD-NMR.

According to Table 3, TD-NMR also performed well when predicting orange total pectin content. A paramagnetic ion, such as iron contained in orange juice [38], may have played an important role in achieving this outcome because it can reduce water relaxation time in some oxidation states [39]. The production of galacturonic acid (HGal) through pectin hydrolysis throughout ripening may reduce ferric ions to ferrous ions, leading to $Fe^{2+}$ solutions featuring longer water relaxation times than their $Fe^{3+}$ counterparts. Studies suggested that the major mechanism for $T_1$ and $T_2$ increased during banana ripening is a reduction of $Fe^{3+}$ ions to $Fe^{2+}$ ions by the galacturonic acid resulting of pectin hydrolysis [39].

### 3.4. PLS prediction models by NIR

Table 4 shows the PLSR models obtained for firmness, peel thickness, and total pectin content using mean center and SNV NIR data. NIR spectra presented high correlations with firmness and total pectin content (*i.e.*, r = 0.87 and r = 0.68, respectively), but poor a correlation with peel thickness (*i.e.*, r = 0.39) for calibration models (Table 4).

Table 5 presents external validation PLSR models for firmness and total pectin content. The correlation coefficients increased while the SEP decreased for this parameter, maintaining the validation.

The low penetration of NIR in the thick tissues of orange peel, which act as a barrier, may explain the lack of correlation between peel thicknesses and NIR data. Although NIR is not able to determine peel thickness directly, it can provide an indirect measurement of such property, which is associated with fruit firmness [40].

NIR has also been used to predict fruit firmness based on differences among scattering and absorption caused by changes in cell wall composition (*i.e.*, pectin and cellulose) during ripening [41]. Changes like cell collapse or the creation of air-filled pores that occur due to the decrease of moisture content during ripening affect the light scattering in fruit tissues [26], resulting in secondary correlations that improve the prediction performance of NIR as for firmness [42,43]. Using two portable NIR spectrometers (Labspec and Luminar) to predict the quality of intact oranges, were found an accuracy level for flesh firmness of 83.9% and 79%, coefficient of cross validation (Rcv) of 0.72 and 0.66, and root mean square error of prediction (RMSEP) equal to 1.05 and 1.39 N, respectively [18].

**Table 5**
External validation of partial least squares regression models using near-infrared data.

| | SEP | R | N |
|---|---|---|---|
| Firmness(N) | 6.22 | 0.92 | 31 |
| Total pectin content (%) | 5.04 | 0.70 | 37 |

SEP = squared error of prediction; r = Pearson correlation coefficient; n = sample universe for validation.

Concerning total pectin content, the absorption of methoxyl groups at 4448.40 $cm^{-1}$ that was clearly observed in previous NIR spectra of pectin [44] may explain the total pectin prediction in intact oranges. Good results were also obtained when evaluating pectin constituents in Japanese pear by NIR [25]. In this case, for the intact fruit spectra, the alcohol-insoluble solids in the fresh weight (AIS in the FW) and the oxalate-soluble pectin content in the AIS (OSP in the AIS) were accurately predicted (for intact fruit spectra: r = 0.93, SEP = 0.62 for AIS in the FW; r = 0.95, SEP = 8.48 for OSP in the AIS).

### 3.5. PLS prediction models by MIR

Initially, the data ranging from 4000 to 3030 $cm^{-1}$ and from 2750 to 1780 $cm^{-1}$ were not used in PLS-MIR analysis because they did not provide any useful information.

Table 6 shows the PLSR models for firmness, peel thickness, and total pectin content using MIR data. The best results for these attributes were obtained with mean center second derivative. MIR spectra presented a high correlation with firmness (r = 0.74) but low correlations with peel thickness (r = 0.33) and total pectin content (r = 0.57) as for calibration models (Table 6).

The external validation shows that PLSR models for firmness maintained suitable correlation coefficients SEP values (Table 7).

Carbohydrates show a high absorbance between 1200 and 950 $cm^{-1}$ in the MIR spectra, which is the fingerprint for polysaccharide [45]. Additionally, MIR spectroscopy is sensitive to the functional groups of these polysaccharides, *i.e.*, hydroxyls, carboxyls, esters, and amides [46]. In this sense, it is possible to explain the high correlation between MIR data and firmness in intact orange, despite having the largest SEP (9.05 N), which in turn may have resulted from the lower penetration of MIR radiation. Even if PLSR model for pectin has not been validated, pectin is one of the polysaccharides that are responsible for orange firmness, making MIR analysis a good methodology to investigate polysaccharides.

Change in pectin methyl esterification degree (MED) during fruit ripening [47] stand out as an important parameter to understand some aspects related to the softening process [45]. Pectin MED features differential localization of absorption bands originated by specific vibrational modes of atom groups in galacturonic acid and its methyl ester.

**Table 4**
Partial least squares regression for calibration models adjusted to near-infrared data.

| | SEV | rVal | SEC | rCal | N |
|---|---|---|---|---|---|
| Firmness (N) | 7.13 | 0.87 | 6.41 | 0.91 | 73 |
| Peel thickness (mm) | 0.63 | 0.39 | 0.55 | 0.62 | 73 |
| Total pectin content (%) | 5.12 | 0.68 | 4.48 | 0.79 | 80 |

SEV and SEC = squared errors of validation and calibration, respectively; n = sample universe; rVal and rCal = Pearson correlation coefficients of validation and calibration, respectively.

**Table 6**
Partial least squares regression for calibration models using mid-infrared data.

| | SEV | rVal | SEC | rCal | n |
|---|---|---|---|---|---|
| Firmness(N) | 9.13 | 0.74 | 7.79 | 0.84 | 73 |
| Peel thickness (mm) | 0.72 | 0.33 | 0.52 | 0.72 | 73 |
| Total pectin content (%) | 6.11 | 0.57 | 5.50 | 0.69 | 83 |

SEV and SEC = squared errors of validation and calibration, respectively; n = sample universe; rVal and rCal = Pearson correlation coefficients of validation and calibration, respectively.

**Table 7**
External validation partial least squares regression models using mid-infrared data.

| | SEP | r | n |
|---|---|---|---|
| Firmness(N) | 9.05 | 0.84 | 32 |

SEP = squared error of prediction; r = Pearson correlation coefficient; n = sample universe for validation.

Therefore, MIR spectroscopy is a suitable means of characterizing pectins [45]. Pectin MED determination in peaches has been correlated with fruit firmness using MIR data [48].

### *3.6. 3.5. Observations concerning TD-NMR, NIR, and MIR methods*

In this study, NIR models for firmness (r = 0.92 and SEP = 6.22 N) were better than those based upon TD-NMR data (r = 0.64 and SEP = 8.27 N). MIR was also efficient to predict orange firmness, but showed a lower Pearson correlation coefficient and a higher SEP than NIR. Despite the lower resolution of NIR spectrum than that of MIR, the greater NIR penetration allowed better correlations with fruit firmness.

TD-NMR allows one to observe the inner environment of fruits. In this context, sugars are the main responsible for viscosity changes in oranges. In this case, the more remarkable effect of sugars over relaxation times may have affected the correlation between firmness variations and TD-NMR signals.

On the other hand, orange peel thickness is related to sugar content. Thus, it provides indirect information on ripening, provided that fruits tend to have higher sugar contents together with thinner peels [49]. This is a possibility explanation for the correlation between TD-NMR signals and orange peel thickness.

TD-NMR showed a higher prediction power than NIR for total pectin content, as indicated by the higher r. TD-NMR has also advantage over NIR because the measurements are performed in intact fruits whereas NIR measurements require the analyses of three regions within the fruit.

## 4. Conclusion

The applicability of TD-NMR, NIR, and MIR spectroscopic techniques combined with PLSR was evaluated for the determination of firmness and other properties related to firmness in intact oranges. The calibration and external validation models developed in this work covered different flowerings and harvests of Valencia orange and, hence, a wide composition range.

NIR and MIR spectroscopies were more efficient in determining orange firmness, whereas TD-NMR decay presented a better prediction capacity as for peel thickness. The penetration of NIR and MIR radiations and typically thick orange peel may have interfered in peel thickness prediction models. The prediction of total pectin content in oranges was carried out through different measurement methods, namely: the intact fruit by TD-MNR as well as specific fruit points by NIR. Regardless of the method, the models were validated for the prediction of total pectin content.

In this work, non-invasive techniques were effectively used to determine properties related to orange quality, which are currently determined by destructive, time-consuming methodologies that involve the use of chemicals. Overall, TD-NMR and NIR PLSR models were more validated compared with MIR model. Nevertheless, all of the non-invasive techniques studied here presented good potential application to determine orange quality attributes, encouraging future studies and further exploitation.

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