



Non-invasive quantification of vitamin C, citric acid, and sugar in ‘Valência’ oranges using infrared spectroscopies

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Abstract Near (NIR) and mid (MIR) infrared spectroscopies have been studied as potential methods for non-destructive analyses of the fresh fruits quality. In this study, vitamin C, citric acid, total and reducing sugar content in ‘Valência’ oranges were evaluated using NIR and MIR spectroscopy with multivariate analysis. The spectral data were used to build up prediction models based on PLS (Partial Least Squares) regression. For vitamin C and citric acid, both NIR ($r = 0.72$ and 0.77 , respectively) and MIR (0.81 and 0.91 , respectively) resulted in feasible models. For sugars determination the two techniques presented a strong correlation between the reference values and analytical signals, with low RMSEP and $r > 0.70$ (NIR: sucrose RMSEP = 12.2 and $r = 0.75$; glucose RMSEP = 6.77 and $r = 0.82$; fructose RMSEP = 5.07 and $r = 0.81$; total sugar RMSEP = 12.1 and $r = 0.80$; reducing sugar RMSEP = 20.32 and $r = 0.82$; MIR: sucrose RMSEP = 9.47 and $r = 0.80$; glucose RMSEP = 6.70 and $r = 0.82$; fructose RMSEP = 5.20 and $r = 0.81$; total sugar

RMSEP = 11.72 and $r = 0.81$; reducing sugar RMSEP = 20.42 and $r = 0.81$). The models developed with MIR presented lower prediction error rates than those made with NIR. Therefore, infrared techniques show applicability to determine of orange quality parameters in a non-destructive way.

Keywords Non-destructive analyses · NIR · MIR · Chemometrics · PLS · Intact fruit quality · Oranges

Abbreviations

CIE	Comission Internationale de l’Éclairage
HPLC	High performance liquid chromatography
FAO	Food and Agricultural Organization
MIR	Middle infrared
NIR	Near infrared
PLS	Partial least squares
r	Pearson correlation coefficient
r_{cal}	Pearson correlation coefficient of the calibration
r_{Val}	Pearson correlation coefficient of the validation
RMSEC	Root mean square error of calibration
RMSEP	Root mean square error of prediction
RMSEV	Root mean square error of validation
SSC	Soluble solids content
USDA	United States Department of Agriculture

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Introduction

Brazil is the world’s largest orange grower, followed by the United States and China (USDA 2019). Brazilian orange production accounts for approximately 25.2% of the total global number (FAO 2019), and most of the produced orange is being used for juice production (USDA 2019). In

the 2018–2019 growing seasons, the world orange production is estimated to reach 54.3 million tons, which will be at the highest levels in 8 years (USDA 2019).

Orange quality, a combination of characteristics and attributes as sugar and organic acid contents, plays an essential role in the degree of acceptability by consumers (Cayuela and Weiland 2010). As well as physical and chemical attributes, nutritional parameters became an important target during the purchase choice. Orange juice is one of the most nutrient-dense fruit juices, two hundred and thirty-seven ml of orange juice provides 21 g of total sugars and 124 mg of ascorbic acid (O'Neil et al. 2012).

In a study to evaluate the effect of the major information on the package of orange juices, the authors found that the nutritional information vitamin C was an important factor for most evaluators, suggesting the concern and preference they have to consume products that provide to them some health benefit (Gadioli et al. 2013).

Ascorbic (vitamin C) and citric acid contents are important criteria to assess the nutritional value of oranges (Jun-fang et al. 2007). Sugars are also significantly important to orange quality, denoting 85% of the soluble solids commonly used for classification purposes during citrus marketing (USDA 2019). Sucrose and the reducing sugars fructose and glucose are the main sugars present in orange juice (Kelebek et al. 2009).

Increasing consumer demand for good quality attributes, such as sweetness and nutritional content, coupled with industry demand for innovative tools for rapid and cost-effective monitoring of quality, has stimulated interest in spectroscopy techniques for citrus fruit quality monitoring and evaluation (Bizzani et al. 2017). Infrared spectroscopy has shown good applicability in fruit quality assessments, the major advantages of near-infrared (NIR) and mid-infrared (MIR) spectroscopies over conventional methods are related to their short analytical times—only a few seconds—as well as their non-destructive character (Nicolai et al. 2014). The conventional analytical methods for determining these parameters are destructive, require much time, chemicals, and trained personnel, besides generating waste.

Infrared spectroscopy can be defined as the study of the interaction of an electromagnetic wave (in the infrared range 14,000 to 50 cm^{-1} , divided into three areas: near IR (14,000–4000 cm^{-1}), mid-IR (4000–400 cm^{-1}), and far IR (400–50 cm^{-1}) with matter (Rodríguez-Saona and Allendorf 2011; Dufour 2009). Orange quality has been analyzed, several times, by using NIR in the last few years (Ncama et al. 2017; Huang et al. 2014; Liao et al. 2014; Liu et al. 2011; Cayuela and Weiland 2010; Xia et al. 2007). Cayuela and Weiland (2010), using two different portable NIR-spectrometers, built SSC and acidity prediction models in a non-destructive way, reaching $R^2 = 0.91$

and 0.83, respectively. Liu et al. (2015), in a study, using NIR to determine SSC, pH, TA (titratable acidity), and vitamin C in Newhall navel oranges, developed satisfactory prediction models, by LS-SVM (Least Squares Support Vector Machine) with a prediction correlation coefficient higher than 0.82. NIR has also been used to quantify vitamin C contents in oranges, showed low RMSEP with $\text{RMSEV} = 3.90 \text{ mg } 100 \text{ g}^{-1}$ (Jun-fang et al. 2007).

MIR, on the other hand, has not been used to analyze whole fruits, due to its low radiation penetration in the sample, the use of which is rather restricted to fruit pulps and juices, e.g., in the determination of total sugars in passion fruit ($R^2 = 0.91$ and $\text{RMSEP} = 7.91 \text{ mg g}^{-1}\text{DW}$) (Oliveira et al., 2014) and apricot pulps (Sucrose, $R^2 = 0.85$ and $\text{RMSEP} = 0.80 \text{ g.}100 \text{ g}^{-1}$; Glucose, $R^2 = 0.87$ and $\text{RMSEP} = 0.26 \text{ g.}100 \text{ g}^{-1}$; Fructose, $R^2 = 0.74$ and $\text{RMSEP} = 0.15 \text{ g.}100 \text{ g}^{-1}$) (Bureau et al. 2009). Nevertheless, the study of the use of MIR to determine quality parameter in intact fruits can show good results when associated with peel chemical composition related to internal quality composition, as essential oil and sugars in the peel that is related to the maturity stage of fruit (Dugo et al. 2011; Ladaniya 2008). One of the advantages of using MIR instead NIR to quantify is the absorption band features; in MIR, the bands are more distinct than in the NIR spectrum, where there are combinations of bands and overtones (Dufour 2009; Pavia et al. 2010).

This study set out to evaluate NIR and MIR techniques as well as the chemometric method named partial least squares (PLS) regression in an effort to develop models for determining the contents of ascorbic acid, citric acid, and sugars in 'Valência' oranges.

Materials and methods

Plant material

Oranges (*Citrus sinensis*) (n = 410) of 'Valencia' cultivar were harvested in three stages (beginning, middle, and crop end) during the 2015 crop season from a commercial orchard in the state of São Paulo, Brazil. The fruits were sanitized and selected by the absence of defects and diseases and stored at $12 \text{ }^\circ\text{C} \pm 1.5$ at relative humidity of 90%. From the total of samples, 80 fruits were used, exclusivity, for standard physical and chemical characterization, 230 oranges were used to develop the sugar PLS models and 150 to organic acids PLS models in the calibration step and external validation.

Physical and chemical characterization

Fresh weight, equatorial diameter, peel color, soluble-solids content, and pH were determined by standard methods using a set of 80 oranges, and each fruit was individually analyzed. These fruits were individually weighed to the nearest 0.01 g in a Marte AS 2000C (São Paulo, Brazil) electronic scale. The values were expressed in grams. The cross-sectional diameter (i.e., the equatorial diameter) was measured to the nearest 0.02 mm with a Mitutoyo digital caliper (Aurora, Illinois).

A HunterLab MiniScan XE Plus colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) was used to evaluate peel color according to the L, a* and b* (color space) system proposed by the *Commission Internationale de l'Éclairage* (CIE). Two-color readings were performed in the equatorial region of each fruit. The results were expressed as a hue angle ($^{\circ}h$).

The soluble solids content (SSC) was determined using an Atago RX-5000cx (ATAGO, Bellevue, WA) refractometer, and the results were expressed as $^{\circ}$ BRIX. The pH measurements were performed by using a QX 1500 QUALSTRON equipment at room temperature ($\pm 24^{\circ}$ C).

Reference analysis for infrared calibration

Ascorbic and citric acid determination

The contents of L-ascorbic and citric acids were determined by high-performance liquid chromatography (HPLC) through a methodology adapted from Kelebek (2009). A Varian liquid chromatograph equipped with an Agilent C18 (2.5 \times 25 mm, 5 μ m) column and an ultraviolet–visible detector set at 254 nm (L-ascorbic acid) and 214 nm (citric acid). Phosphate buffer (0.1 M) at pH 2.5 was used as the mobile phase with a flow rate of 1.0 mL min⁻¹, and the injection volume was 20 μ L. The concentration of each acid was determined by using a calibration curve that was prepared with pure L-ascorbic and citric acid standards (Sigma Aldrich, St. Louis, MO).

After the juice extraction, the samples were brought to a known volume with 3% (w/v) metaphosphoric acid, filtered through a disposable filter made up of hydrophilic Teflon (porosity of 0.45 μ m), and protected from light to prevent organic acid degradation. The analyses were performed, individually, in each fruit in duplicate.

Total and reducing sugars

Total and reducing sugars were quantified by HPLC with a methodology adapted from Kelebek (2009), using a Varian liquid chromatograph equipped with an HPX 87H column and a refractive index (RI) detector. The column was

maintained at room temperature (25 $^{\circ}$ C). Sulfuric acid (H₂SO₄ 0.5 mM) was used as the mobile phase with a flow rate of 0.6 mL min⁻¹. 20 μ L of each sample was injected into the system. Juice samples of each fruit were extracted and stored at -28° C. Samples were thawed, diluted to 1:10 ratio with ultrapure water (Milli-Q®), and filtered through a disposable filter made up of hydrophilic Teflon (porosity of 0.45 μ m) before the analyses. Standards comprised 99%+ pure sucrose, 99.5%+ pure D-glucose, and 99% + pure D-fructose (Sigma Life Science).

Near-infrared (NIR) spectroscopy

The oranges reflectance NIR spectra were collected using the Spectrum 100 N spectrometer (Perkin-Elmer Corp., Norwalk, CT). The spectral resolution was 32 cm⁻¹ and 32 spectra were co-added to improve the signal to noise ratio over the 4000–10,000 cm⁻¹ range. The analysis was performed in each fruit, individually, in the equatorial region, and the average of these three spectra was used to build up the models.

Mid-infrared (MIR) spectroscopy

The MIR attenuated total reflectance (ATR) spectra were acquired in an Agilent Cary 630 FTIR spectrometer at wavenumbers ranging from 650 to 4000 cm⁻¹, with a resolution of 16 cm⁻¹, and using 64 scans per sample. The analysis was performed in four equidistant equatorial positions of each orange. The average of four spectra was used to build up the models.

Data processing

The chemometric analyses were performed using the Origin 8.1 (OriginLab, Northampton, MA, USA) and Pirouette v.4.5 (Infometrix, Inc. Bothell—WA) softwares. NIR spectra were mean-centered, processed with Standard Normal Variation (SNV), and Savitzky-Golay first derivative method with a twenty-one points window. The MIR spectra were mean-centered, normalized between 1 to 0, and with Savitzky-Golay first derivative method with five points of window.

The partial least squares (PLS) regression models we constructed using the NIR and MIR data as independent variables (X matrices) and the data obtained with the reference methods as dependent variables (Y matrices), 70% of the total orange set, used to build the models, were used to develop the calibration models, and internal validation and 30% were used in the external validation procedure.

The evaluation of the models was taken from the analysis of the Pearson correlation coefficient (r) of the calibration (r Cal), validation (r Val) and prediction (R), as

well as the root-mean-square errors of calibration (RMSEC), validation (RMSEV), and prediction (RMSEP).

Results and discussion

Physical and chemical orange characterization

Table 1 shows the average and variation range of the mass, diameter, skin color, SSC, and pH values of the orange samples. The considerable variation in mass, SSC and pH can be related to the uses of orange from four flowerings, different nutritional and hormonal states of the plant, and environmental conditions during plant growth (Tadeo et al. 2008).

The mass values are similar to those reported to Valencia' oranges by Arruda and colleagues (2011) in Brazilian orchards and those described by Magwaza et al. (2013) when evaluating the quality of 'Valencia' oranges using NIR spectroscopy. On the other hand, Bai et al. (2016) obtained values up to 153 g per fruit in US orchards, which is quite different from our findings. The range of the values of the equatorial diameters allows the classification of oranges in classes 3 (80–93 mm), 6 (70–80 mm), and 12 (56–63 mm), according to the CODEX standard (CODEX 2004).

The external color values agree with the °hue reported by Arruda et al. (2011) and Miranda et al. (2015). The °hue was used as a parameter to monitor the peel color and could vary from 0° to 360°. Angles between 60 and 150 indicate fruits with greenish to yellowish skin, respectively (McGuire 1992). In this study, was used fruits from 76° to 108.62 °hue, covering greenish to yellowish oranges (showing different stages of ripeness).

The soluble solids content, which denotes an indirect measure of juice sweetness (Kader 2002), presented ranges and averages similar to those found in the literature (Cayuela and Weiland 2010; Tadeo et al. 2008). Particularly for orange juice, pH is an important quality parameter. Along with titratable acidity, pH suggests fruit acidity. It was observed that the average pH was 3.47, pH values

higher than 3.5 suggest less acidic fruits, while those lower than 3.5 suggest acidic fruits (Ting 1969).

Reference analysis for infrared calibration

Table 2 shows the average, standard deviation, and data range values for vitamin C, citric acid, sucrose, glucose, fructose, total, and reducing sugar that was used to calibrated infrared models. As in the routine physical–chemical characterization, the reference analysis results show a wide range of values for each parameter. The largest variations, six and tenfold, were observed in vitamin C and citric acid, and the smallest variation (threefold) was observed in the glucose/ fructose content. Such large variation is essential for the models as the regression must be calibrated in the full range of values found in the samples (Nicolai et al. 2014).

NIR and MIR spectra

The spectra of two distinct samples (L1 and L2) are shown in Figs. 1 and 2. L1 presents a low sugar content (sucrose: 24.59 g L⁻¹; glucose: 29.70 g L⁻¹; fructose: 27.98 g L⁻¹; total: 82.28 g L⁻¹; and reducing: 57.69 g L⁻¹), and L2 presents high levels of sugars (sucrose: 54.79 g L⁻¹; glucose: 50.08 g L⁻¹; fructose: 48.90 g L⁻¹; total: 153.78 g L⁻¹; and reducing: 98.98 g L⁻¹).

Figure 1 shows the orange NIR reflectance spectra after preprocessing (A) with a mean center, standard normal variation (SNV), and Savitzky-Golay first derivative method (SG) with a twenty-five points window and the raw spectra (B). SNV has been often used in NIR to remove baseline and path length problems. SG method has been applied to enhance the resolution of overlapped peaks, baseline shift, multiplicative interference from dispersion, particle size, and change in light distance (Cen and He 2007). Therefore, these data preprocessing procedures are

Table 1 Results of the characterization of orange samples

	Mean ± sd	Interval
Mass (g)	188.04 ± 41.28	93.72–282.23
Diameter (mm)	71.17 ± 4.18	63.80–80.38
Skin color (°hue)	91.99 ± 10.07	76.00–108.62
SSC (° BRIX)	8.18 ± 1.03	4.80–11.48
pH	3.47 ± 0.39	3.47–4.23

SSC = soluble solids content; sd = standard deviation

Table 2 Average, standard deviation, and data interval of the vitamin C, citric acid, sucrose, glucose, fructose, total and reducing sugar

Parameter	N	Average ± sd	Interval
Vit C (mg L ⁻¹)	230	412.52 ± 146.54	175.6–1074.6
Citric acid (g L ⁻¹)	230	11.56 ± 6.25	4.10–40.25
Sucrose (g L ⁻¹)	150	51.24 ± 18.38	21.80–113.07
Glucose (g L ⁻¹)	150	44.14 ± 11.86	25.04–77.09
Fructose (g L ⁻¹)	150	40.80 ± 8.59	24.15–65.60
Total (g L ⁻¹)	150	134.47 ± 36.18	72.30–219.16
Reducing (g L ⁻¹)	150	84.22 ± 19.97	49.51–137.43

sd = standard deviation; N = sample universe

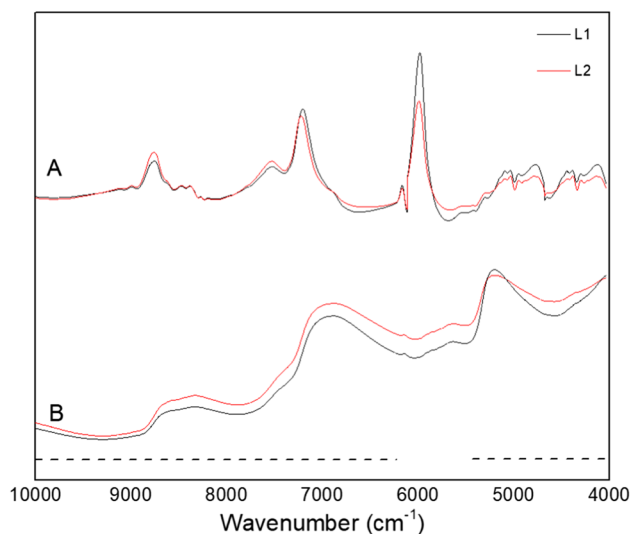


Fig. 1 NIR spectra submitted to SNV and first derivative (25) (a); original NIR spectra (b); red and black lines are high and low sugar content samples, respectively; Dashed line: spectrum variables used

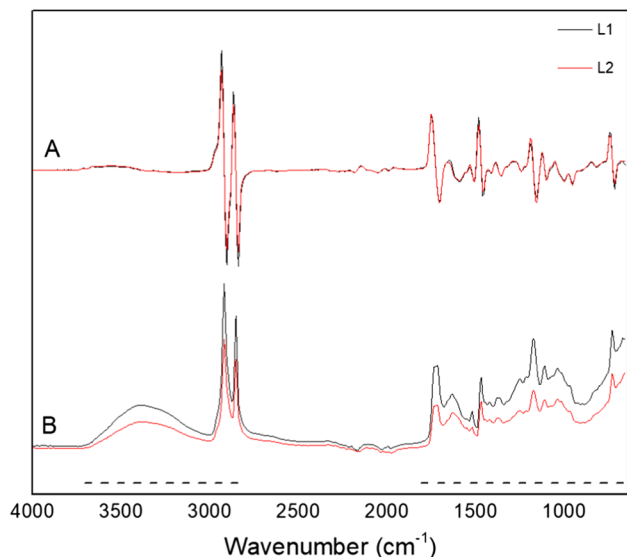


Fig. 2 Original MIR spectra (a); b)MIR spectra submitted to SNV and first derivative (25); Dashed line: spectrum variables used. L1 orange with low sugar content and L2 with high levels of sugar

commonly used to minimize or eliminates NIR baseline variation and light scattering and overlapped peaks and consequently to the development of more accurate models (Cen and He 2007). Spectral preprocessing techniques also helps to remove any irrelevant information, which cannot be handled properly by the regression techniques (Nicolai et al. 2014).

After the preprocessing and transformations of spectra, it was noticed that the difference in baseline was corrected; in addition, with the application of the first derivative, some peaks, previously absent, were evidenced. Correction

of these problems is advantageous and important for the chemometric step of developing the correlation models with the wanted physicochemical parameters.

Therefore, the raw (1A) and processed (1B) NIR spectra show three strong bands at 5000, 7000, and 8500 cm^{-1} that have been assigned to OH, OH, and CH stretching vibration, respectively (Lin et al. 2009; Bizzani et al. 2017). The PLS models using NIR spectra were built without data from 6400 to 5600 cm^{-1} because this region is characterized by combination bands of amide groups, present in protein molecules.

Figure 2 shows the MIR spectra of the same two oranges samples. A is the original spectra, and B is the pre-processed with mean-centered, normalization from 1 to zero, SG first derivative method (SG) with five points of window. In both spectral data sets (NIR and MIR), PLS models were developed with raw and processed spectra. However, those developed with the raw data did not show a high correlation with the quality parameters due to defects such as light scattering, baseline difference, etc.

The strong and broadband from 3200 to 3600 cm^{-1} in MIR spectra (Fig. 2a) oranges has been assigned to the O–H group of water, organic acids, and sugars (Bizzani et al. 2017; Huang et al. 2009). The sharp bands between 3000 and 2750 cm^{-1} have been assigned to C–H vibrations, which are present in most of the organic molecules but are strong in molecules, such as fatty acids (Bicudo et al. 2005) and essential oils that are present in large amount in orange peel. Limonene is the main component of the essential oils found in citrus peel (Kader 2002). In general, the content of peel oil increases with fruit maturity (Ladaniya 2008), with some exceptions as Limonene level that decreases at the beginning and end of the orange harvest season (Dugo et al. 2011). Therefore, harvest time is a critical parameter that significantly influences the essential oil composition in citrus (Bourgou et al. 2012). There are no studies that report a correlation between oils' peel composition and the quality of oranges. Moreover, some results indicated that sugar concentration of the orange peel, similar to what occurs in the juice, changes during the maturation time (Ladaniya 2008), accumulating with maturity. Sucrose and glucose are the predominant sugars in the early stages of peel maturation.

The band in 1730 cm^{-1} has been assigned to the C=O groups found in organic acids, esters, aldehydes, and ketones (Bicudo et al. 2005). The interval between 1790 and 706 cm^{-1} has been associated with the stretching of C=O and C=C groups (Yang and Irudayaraj 2002). In contrast, C–C and C–O stretching groups predominate at bands between 1500 and 900 cm^{-1} (Pavia et al. 2010). Bureau et al. (2019) observed that the region between 1500 and 900 cm^{-1} is the most adequate to quantify sugars. When analyzing vitamin C solutions, Yang and Irudayaraj

(2002) observed a strong correlation between the bands at 3500–3000 cm^{-1} and 1750–1000 cm^{-1} with different acid concentrations.

The PLS models using MIR spectra were built without data from 3656 to 4000 cm^{-1} and from 2777 to 1773 cm^{-1} regions because they do not present any band, only noise.

PLS models for vitamin C and citric acid Using NIR and MIR data

Table 3 presents the results of Pearson correlation coefficient of the calibration (r_{Cal}), Validation (r_{Val}) as well as the root-mean-square errors of calibration (RMSEC), validation (RMSEV) for the PLS models developed with NIR and MIR data for the determinations of the parameters vitamin C and citric acid.

The PLS analyses were performed with 230 oranges using 10 factors (F), except the NIR analyses of citric acid that was 8. The choice of the number of factors was made from the observation of RMSEC and RMSEV values, where the number of suitable factors showed the smallest error values (Geladi and Kowalski 1986).

According to Varmuza and Filzmoser (2016), models with r above 0.7 and below 0.9 presents a strong correlation, and models with r between 0.4 e 0.7 are considered with moderate correlation. Therefore, vitamin C and citric acid models developed with NIR and MIR data resulted in models with a strong correlation. However, the r_{Val} for

Table 3 Correlation coefficients and errors of PLS models developed with NIR and MIR data for the determinations of the quality parameters

Parameter	Method	RMSEV	r_{VAL}	RMSEC	r_{CAL}
Vit C (mg L^{-1})	NIR	107.2	0.72	89.3	0.82
	MIR	103.4	0.78	89.7	0.82
Citric acid (g L^{-1})	NIR	4.1	0.77	3.7	0.83
	MIR	1.7	0.91	1.6	0.92
Sucrose (g L^{-1})	NIR	11.0	0.77	8.9	0.87
	MIR	7.0	0.90	6.5	0.91
Glucose (g L^{-1})	NIR	7.8	0.76	6.0	0.88
	MIR	7.7	0.74	6.7	0.83
Fructose (g L^{-1})	NIR	5.2	0.80	3.9	0.90
	MIR	6.4	0.70	5.5	0.79
Total (g L^{-1})	NIR	21.9	0.79	17	0.89
	MIR	13.8	0.72	11.9	0.81
Reducing (g L^{-1})	NIR	12.6	0.78	9.3	0.90
	MIR	23.6	0.75	20.1	0.83

NIR = near-infrared; MIR = mid-infrared RMSEV and RMSEC = root-mean-square errors of validation and calibration, respectively; r_{Val} and r_{Cal} = Pearson's correlation coefficients of validation and calibration, respectively

MIR was higher than NIR for vitamin C and citric acid, mainly to citric acid with a correlation coefficient of validation step above 0.90. MIR also shows lower validation error (RMSEV) to the citric acid PLS model, 1.7 g L^{-1} , indicating a great and accurate method to determine this attribute.

Table 4 presents the results of the root-mean-square errors of prediction (RMSEP) and r for external validation with 99 oranges using 9 and 8 factors for vitamin C and citric acids, respectively. Both techniques show $r > 0.7$ for both compounds. Like in the internal validation, MIR shows higher r and lower RMSEP than NIR.

The lower performance of NIR is known to present overburdened spectra containing many absorption combinations with poorly defined bands. This might have hindered the correlation between the signals and the quality parameters (Bobelyn et al. 2007; Nicolai et al. 2014). Even so, the models developed with NIR presented a strong correlation and low mean errors. Magwaza et al. (2013) evaluating the use of NIR in determining vitamin C in 'Valência' oranges reported values of RMSEP close to that found in this study, 90 mg L^{-1} on average. However, the correlation coefficient values were below 0.60.

The use of NIR technique for determining citric acid and vitamin C contents in intact oranges has been evaluated in other studies, such as that carried out by Miyamoto et al. (1998) on the quantification of citric acid in whole oranges through NIR spectroscopy, in which the developed PLS

Table 4 Statistical outcomes of the PLS prediction models for MIR analytical signals for vitamin C, citric acid, sucrose, glucose, fructose as well as total and reducing sugars vitamin C and citric acids, respectively, NIR and MIR data

Parameter	Method	RMSEP	r
Vit C (mg L^{-1})	NIR	94.9	0.71
	MIR	75.1	0.81
Citric acid (g L^{-1})	NIR	3.8	0.75
	MIR	3.01	0.83
Sucrose (g L^{-1})	NIR	12.2	0.75
	MIR	9.4	0.80
Glucose (g L^{-1})	NIR	6.7	0.82
	MIR	6.7	0.82
Fructose (g L^{-1})	NIR	5.0	0.81
	MIR	5.2	0.81
Total (g L^{-1})	NIR	12.1	0.80
	MIR	11.7	0.81
Reducing (g L^{-1})	NIR	20.3	0.82
	MIR	20.4	0.81

NIR = near-infrared; MIR = mid-infrared; RMSEP = root-mean-square errors of prediction; r = Pearson's correlation coefficients of prediction; n = sample universe

models showed a Pearson's correlation coefficient of 0.83. In the investigation done by Pissard et al. (2013), NIR-based models for the determination of vitamin C in apples were developed and led to $r = 0.89$.

MIR technique showed the best models, as indicated $RMSEP = 75.1$ and $r = 0.81$ for vitamin C as well as $RMSEP = 3.0$ and $r = 0.83$ for citric acid. For MIR, there are no other studies on its use for non-destructive analyses of oranges. The results of this study show there is a correlation between organic acid concentration in the juice with some compounds of orange peel, most probably essential oil that during the maturation change its concentration and composition. Even with a low MIR radiation penetration, it was observed a strong correlation and a low prediction error.

PLS models of sugar contents

Table 3 presents the results of Pearson correlation coefficient of the calibration (r_{Cal}), Validation (r_{Val}) as well as the root-mean-square errors of calibration (RMSEC), validation (RMSEV) for the PLS models developed with NIR and MIR data for the determinations of the sucrose, glucose, fructose, total and reducing sugars, using 150 samples and 6 to 10 factors.

Therefore, the models developed with NIR and MIR data for sugars (Table 2) resulted in models with a strong correlation. However, the r_{Val} for NIR was higher than MIR for almost all measurements, except to sucrose where r_{Val} value was 0.90. On the other hand, the RMSEV values were lower MIR for sucrose, glucose, and total sugars and NIR RMSEV values were lower for fructose and reducing sugars.

Table 4 presents the results of the root-mean-square errors of prediction (RMSEP) and r for external validation with 45 oranges using 5 and 8 factors for sucrose, glucose, fructose, total, and reducing sugars. The models present a strong correlation with $r > 0.70$. In addition, the RMSEP values of each quality parameter were similar for both methods and were lower than those found in the internal validation (Table 3). Even though the use of MIR to assess the quality parameters of whole fruits is still limited, this technique showed promising results. This finding suggests the power of this model in separating a sample containing high sucrose concentrations from those having low sucrose concentration.

Conclusion

The applicability of spectroscopic techniques in NIR and MIR range combined with PLS regression was evaluated for the determination of sugars and organic acids contents in intact oranges. The results demonstrated the potential of NIR and MIR-techniques to determine orange quality in a

non-destructive way. Mid infrared shown best PLS models, with higher correlation coefficients and lower errors to all parameters, except fructose and reducing sugars. Therefore, it can be an alternative for quality control of raw materials for several food products. Further studies to optimize the prediction models should be conducted to reduce RMSEP values and tests to prove the correlation between the essential oil composition of orange peel and its juice quality parameters.

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