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DETERMINATION OF INDIVIDUAL SUGARS IN PASSION FRUIT USING NIR AND MIR SPECTROSCOPIC METHODS

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ABSTRACT – In this work, NIR and MIR spectroscopy was investigated and compared for predicting individual sugars (sucrose, glucose and fructose). Spectra of 56 samples of lyophilized passion fruit were collected using an integrating sphere in NIR range and attenuated total reflectance accessory in MIR range. Spectral and reference data were analyzed by principal component analysis. Partial least square regression (PLSR) was used to establish calibration models. MIR technique was better than the NIR technique for glucose ($R^2_v = 0.942$), fructose ($R^2_v = 0.855$) and sucrose ($R^2_v = 0.818$).

1. INTRODUCTION

Trends in analytical chemistry are towards simple and less time consuming analytical methods, through advances in new technologies, automation, development of qualitative and screening methodologies, and the increasing use of chemometrics (Karoui *et al.*, 2006). Infrared spectroscopy has been proposed as an interesting alternative to traditional methods due to its rapidity, simplicity, cost effectiveness and potential for routine analysis if proper calibration and validation steps were developed. Besides, it does not require special skilled operators (Karoui *et al.*, 2006; Tavakolian *et al.*, 2013).

Compound identification by infrared spectroscopy is based on the molecules property to absorb infrared light and experience a wide variety of vibrational motions characteristic of their composition. Mid-infrared (MIR) spectra (4000 – 400 cm^{-1} range) provide information from frequencies of fundamental molecular vibrations, while near-infrared (NIR) (2500 – 700 nm range) spectra contain information from overtones and their combinations (Shiroma and Rodriguez-Saona, 2009). Compared to MIR, light can better penetrate into the matter and favor applications in bulk or intact samples in NIR range, requiring little or no sample preparation. As light diffusion is much greater in NIR range, spectra are more sensitive to factors that influence light diffusion as physical structure and presence of water in the sample (Williams and Norris, 1987). Besides, NIR spectrum is much more encumbered due to abundance of combinations and overtone bands and as a consequence, bands are less specific in the NIR range compared to MIR. Therefore, MIR spectroscopy is highly sensitive to chemical composition of samples compared to NIR (Bellon-Maurel and McBratney, 2011). These different features of NIR and MIR spectroscopy suggest that one of them can be more adequate to each application.

Assuring fruit quality is gaining attention in recent years. Several reports are available in the literature concerning variables that represent quality in fruits (Scibisz *et al.*, 2011; Tavakolian *et al.*, 2013; Ribeiro *et al.*, 2013; Oliveira *et al.*, 2014). Among the most important variables are the sugar contents, due to their role in fruit quality. Analytical techniques have been developed to evaluate this parameter, however, traditional methods are not well adapted to on-line measurements and require an expensive and time consuming sample preparation. Most of them are based on solvent extraction followed by high performance liquid chromatography (HPLC) separation and/or colorimetric and enzymatic analyses (Kelebek *et al.*, 2009).

Previous researches have demonstrated the potential of MIR and NIR technology for quantitative characterization of fruits. Pedro and Ferreira (2007) applied the same methodology (NIR) to predict sugar content and acidity in tomato products. Bureau *et al.*, (2009) used the MIR spectroscopy to determine sugar, organic acid and polyphenols contents in apricot and apples. The Passion fruit species belonging to the *Passifloraceae* family are mostly native at Latin America and are much used in juices, jams and yogurts manufacturing. In this context, the aim of this work was to assess the performance of NIR and MIR spectroscopy in determining individual sugar, in passion fruits in different ripening stages.

2. MATERIALS AND METHODS

2.1. Selection and preparation of passion fruit samples

Fifty-six yellow passion fruits (*Passiflora edulis f. flavicarpa*) in different ripening stages were collected in south of Brazil. Pulp was separated from the seed by filtration and then lyophilized. Lyophilized pulp was used in order to eliminate interferences caused by physical features of the fruit, as a recent report of our research group has pointed out (Oliveira *et al.*, 2014). Each sample was characterized in its spectral and biochemical analysis.

2.2. Spectra acquisition

A multi-purpose analyser (MPA) spectrometer (Bruker Optics, Wissembourg, France) in 2500 to 800 nm range was used for collecting NIR spectra in reflectance mode. The instrument was equipped with an integrating sphere to provide diffuse reflectance measurements and a TE-InGaAs detector. MPA was completely software-controlled by OPUS software Version 5.0, provided by Bruker Optics. Thirty-two scans were averaged for each sample spectrum. It was acquired with a 2 nm spectral resolution. Lyophilized pulps were transferred into glass vials and placed on automated 30-position sample wheel, each position corresponding to an 18 mm diameter hole. The average spectrum obtained with two measurements of powder from different aliquots was used in calculation.

MIR spectra were collected at room temperature with a Tensor 27 FTIR spectrometer (Bruker Optics, Wissembourg, France) equipped with a single-reflectance horizontal ATR cell with a diamond crystal (Golden Gate, Bruker Optics) and a deuterated triglycine sulfate (DTGS) detector. Lyophilized and homogenized samples were placed at the surface of the diamond crystal and were pressed with a system press tip flap. Samples were scanned at wavenumbers ranging from 4000 to 600 cm^{-1} and

corrected against the background spectrum of air. Thirty-two scans were averaged for each sample spectrum. Two spectra, from two different aliquots of the powder, were performed for each sample. Average spectrum was used in model development.

2.3. Determination of soluble solids content

Sugars (glucose, fructose, sucrose) were quantified by enzymatic measurements with kits for food analysis (Boehringer Mannheim Co., Mannheim, Germany). The method was adapted on a 96 well-microplate (Vermeir *et al.*, 2007) using a spectrophotometer (FLX-Xenius, SAFAS, Monaco) equipped with an automatic injection device. Sugar contents were expressed in mg g⁻¹ DW (dried weight).

2.4. Multivariate calibration

MatLab software package (version 6.5, Mathworks USA.) and Origin 6.1® (OriginLab Inc., Northampton, USA) were used for chemometric treatment of data. PCA (principal component analysis) was initially performed for all samples (56 samples) to define calibration (80% samples) and external validation (20% samples) groups

In this study, different pretreatments, including mean centering, multiplicative scatter correction (MSC), first-derivative and smoothing, were applied. Quantification models for sucrose, glucose, fructose contents were developed using PLS regression (Coury and Dillner, 2008). Calibrations were performed in order to avoid overfitting data and obtain a reliable model. PLS regression and leave-one-out cross-validation were used in calibration step. Cross-validation estimated the prediction error by splitting all samples into two groups. Leave-one-out cross-validation (on 80% of data) involves using a single observation from the original sample as validation data and the remaining observations as training data. This process was repeated until all the samples had been used once in the validation set (Xie *et al.*, 2007).

To evaluate the results, root mean square error of cross-validation (RMSECV), root mean square error of prediction (RMSEP), correlation coefficient of calibration (R^2_{cv}) and of external validation (R^2_v) were considered.

3. RESULTS

3.1. Chemical and Spectral diversity

Table 1 shows sugars contents determined in lyophilized pulp of passion fruit samples. According to these data, sucrose is the main sugar, presenting the highest content in passion fruit. Haard and Chism (1996) pointed out that main sugars in passion fruits are the three simple sugars: sucrose, glucose and fructose and that sucrose is the main sugar, followed by glucose and fructose. Moreover, these authors affirm that sugars represent about 80% of the soluble solids of passion fruit pulp.

Table 1 – Quality traits (mg g⁻¹ DW) of passion-fruit samples.

Parameter	Range	Median	Standard deviation
Glucose	23.64 - 217.38	96.34	±38.98
Fructose	28.37 - 240.03	97.59	±46.19
Sucrose	40.43 - 396.22	293.92	±65.03

MID and NIR mean spectra of passion fruit without any treatment are shown in the Figures 1a and b, respectively. Full spectra presented characteristic peaks. (Bureau *et al.*, 2012; Bureau *et al.*, 2009; Ruiz *et al.*, 2008). In Figure 1a, MIR spectrum of lyophilized passion fruit pulp showed characteristic absorption zones in 1500–900 cm⁻¹ corresponds to the major components, particularly sugars and organic acids (Bureau *et al.*, 2009; Ruiz *et al.*, 2008). Variation around the peak at 1200–900 cm⁻¹ can be associated with C–H group and the peak at 1500–1200 cm⁻¹ can be associated with C–O (Skoog *et al.*, 2002), and may be related to the organic acids and sugars contents (Scibisz *et al.*, 2011). Regarding to the NIR spectrum (Figure 1b), four broad absorption peaks can be seen around 1190, 1450, 1790, and 2380 nm wavelengths. The peak at 1190 nm is in agreement with the second and third C–H overtone regions and the 1790 nm peak overlaps with the first C–H overtone region, both associated to sugar solutions (Skoog *et al.*, 2002). The slight peak at 2300 nm falls within the region associated with C–H and O–H-combinations grouping.

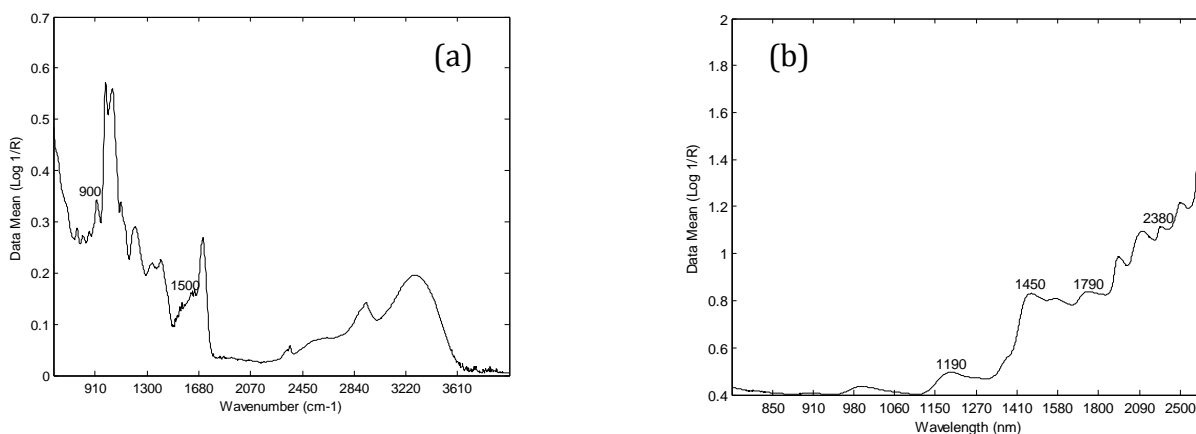


Figure 1 – Spectrum of lyophilized passion fruit pulp: MID (a) and NIR (b)

PCA performed with MID and NIR spectral data using mean center processing is presented in Figure 2. This analysis allowed detecting anomalous spectra and to identify specific sets of samples. In PCA sample map for MID spectra (Figure 2a), the first PC-score (PC1) discriminated the fruits in two sets. The first set, marked with a circle, corresponds to less sweet fruits, and the second set,

market with a square, to the sweetest fruits, with sugar concentration from 300.18 to 762.89 mg g⁻¹ DW.

PCA for NIR spectra (Figure 2b) also identified sample 39, that is a high acid content sample, on the left side and sample 30, a high sugar content sample, on the right side. The specificity of bands is less in NIR range compared to MID (Bellon-Maurel and McBratney, 2011). Thus, in Figure 2b, there was no group separation and the difference among samples can be observed through a straight line, which presented an increasing in acid content. More specifically, sample 30 presented a low acid concentration and sample 10, a high acid concentration.

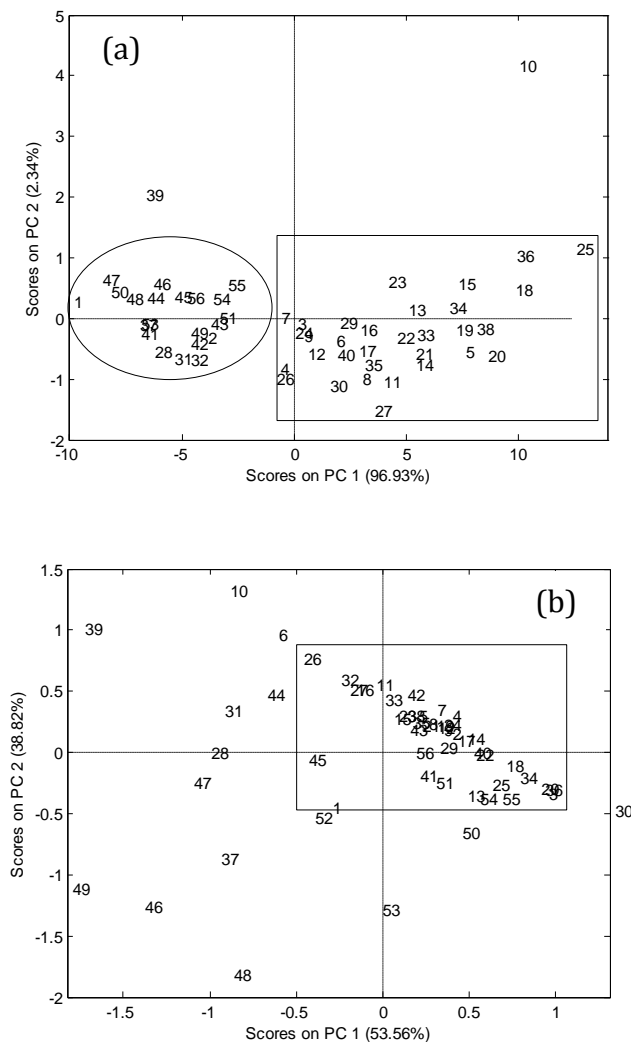


Figure 2 – PCA performed with MID (a) and NIR (b) spectral data.

3.2. Quantification of individual sugars

A summary of prediction performance of calibration models developed for each chemical parameter in NIR and MID spectral ranges is shown in Table 2.

Table 2 - Performance of PLS regression using the MID (cm^{-1}) and NIR (nm) spectral ranges for non destructive composition assessment (mg g^{-1} DW) of freeze-dried passion fruit pulp.

Parameter	Spectral range	Outliers	LV*	Wavelength range	R^2_{cv}	RMSECV ^a	R^2_v	RMSEP %
Glucose	MID	2	5	4000-600	0.945	13.126	0.941	8.712
	NIR	2	6	2500-1100	0.876	21.102	0.862	9.645
Fructose	MID	2	5	4000-600	0.934	15.773	0.855	8.340
	NIR	1	5	2500-1100	0.821	21.343	0.756	11.685
Sucrose	MID	2	6	4000-600	0.889	28.531	0.818	5.825
	NIR	1	6	2500-1170	0.722	46.191	0.691	7.838

*LV: number of latent variables

The best results obtained for glucose ($R^2_v = 0.941$; RMSEP = 8.712%), fructose ($R^2_v = 0.855$; RMSEP = 8.340%) and sucrose ($R^2_v = 0.818$; RMSEP = 5.825%) were obtained with MID spectroscopy. Both reasons (spectral specificity and concentration range) contributed to the relative ease and precision of the prediction in the MID range. The number of latent variables for the models were very close. PLS regression models appeared to have an excellent ability to estimate sugars concentration, based on the very high R^2_v and low RMSEP results, which was in agreement with previous works (Xie *et al.*, 2009; Rambla *et al.*, 1997).

4. CONCLUSION

The applicability of spectroscopic techniques in MID and NIR range combined with PLS regression was evaluated for the determination of individual sugars, in lyophilized passion fruit pulp. Calibration models developed in this work covered different maturation stages of passion fruit and thus a wide range of compositions.

In this work, MID spectroscopy was considered better for determination quality parameters in passion fruit. This fact can be attributed primarily because MID spectroscopy contains wavelengths for fundamental rotational molecular vibration, while overtone and combination bands of fundamental

transition are associated with near infrared spectrum, making it less reproducible and specific. A second reason that can be assigned is that mid-infrared region is highly sensitive to precise chemical composition of samples compared to near infrared technique.

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