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Rapid analysis of sugars in fruit juices by FT-NIR spectroscopy

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Abstract

A simple analytical procedure using FT-NIR and multivariate techniques for the rapid determination of individual sugars in fruit juices was evaluated. Different NIR detection devices and sample preparation methods were tested by using model solutions to determine their analytical performance. Aqueous solutions of sugar mixtures (glucose, fructose, and sucrose; 0–8% w/v) were used to develop a calibration model. Direct measurements were made by transflection using a reflectance accessory, by transmittance using a 0.5-mm cell, and by reflectance using a fiberglass paper filter. FT-NIR spectral data were transformed to the second derivative. Partial least-squares regression (PLSR) was used to create calibration models that were cross-validated (leave-one-out approach). The prediction ability of the models was evaluated on fruit juices and compared with HPLC and standard enzymatic techniques. The PLSR loading spectra showed characteristic absorption bands for the different sugars. Models generated from transmittance spectra gave the best performance with standard error of prediction (SEP) $< 0.10\%$ and R^2 of 99.9% that accurately and precisely predicted the sugar levels in juices, whereas lower precision was obtained with models generated from reflectance spectra. FT-NIR spectroscopy allowed for the rapid (~ 3 min analysis time), accurate and non-destructive analysis of sugars in juices and could be applied in quality control of beverages or to monitor for adulteration or contamination.

Keywords: Sugars; Fruit juices; FT-NIR spectroscopy; Multivariate analysis

1. Introduction

The determination of individual sugar content in fresh fruits and vegetables and their juices is an important chemical analysis carried out to evaluate quality and to detect adulteration or contamination. Analytical techniques such as liquid chromatography using different separation techniques (reverse-phase, ion-exclusion, ion chromatography) and detectors (refractive index, UV absorption, amperometric), thin-layer chromatography, and gas chromatography have been commonly used for qualitative and quantitative analyses of fruit juices.[1] While chromatographic techniques are very accurate, they are time-consuming and require extensive sample preparation. Sugar analyses carried out by enzymatic methods are specific, rapid and reproducible,[2] however the analyses require single determinations for each compound, which results in time-consuming procedures and high cost of analysis.[3]

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Near-infrared spectroscopy (NIR) is a non-destructive, fast and accurate technique for measurement of chemical components based on overtone and combination bands of specific functional groups.[4,5] The NIR bands are 10–100 times less intense than the corresponding mid-infrared fundamental bands. This enables direct analysis of samples that are highly absorbing and strongly light scattering without dilution or extensive sample preparation.[5,6] Nevertheless, NIR measurement of aqueous systems has been difficult because of interference from broad vibrational bands of water.[7] The application of NIR transmittance analysis of raw products such as sugarcane and beet juice/syrups for pol (sucrose) and °Brix is a conventional analytical technique for quality monitoring in the sugar industry.[8] Lanza and Li[9] reported the application of NIR spectroscopy for the direct analysis of total sugar content in fruit juices. However, they concluded that it was not possible to determine individual sugars with acceptable accuracy or precision by using the transmission mode with a quartz cell pathlength of 2.2 mm. Giangiacomo and Dull[10] developed NIR models based on transmittance measurements that predicted individual sugars (sucrose, glucose, and fructose) in aqueous mixtures with a standard error of prediction of 0.35–0.69. Improved sensitivity and accuracy for the quantitative analysis of individual sugars in juices have been accomplished by placing the liquid sample on a fiberglass support, eliminating the water and measuring the dry extract by diffuse reflectance spectroscopy.[11,12]

Advances in Fourier transform NIR (FT-NIR) spectroscopic instrumentation and multivariate data analysis techniques have had significant impact in the determination of changes in food composition. FT-NIR improves spectra reproducibility and wavenumber precision,[13] which can minimize the effects of solvent interference. Multivariate statistics have provided chemometric tools such as principal components analysis (PCA) and partial least-squares regression (PLSR) methods that are able to model relationships between large numbers of dependant variables having extremely complex variations (such as NIR spectra) and independent variables (such as chemical concentrations).[14] PLSR has been particularly successful in developing multivariate calibration models for NIR spectroscopy because it uses the concentration information (Y-variable) actively in determining how the regression factors are computed from the spectral data matrix (X), reducing the impact of irrelevant X-variations in the calibration model.[15,16] This capability provides a more information-rich data set of reduced dimensionality and eliminates data noise that results in more accurate and reproducible calibration models.[17]

The objective of this study was to develop methodology for the rapid identification and quantification of individual sugars in fruit juices using FT-NIR spectroscopy and multivariate methods, based on a calibration set of aqueous standard solutions. Different NIR sampling techniques that included reflectance, transflection and transmittance were tested by using model solutions to determine their analytical performance.

2. Materials and methods

Sample preparation.—Analytical grade D-glucose, D-fructose and sucrose (Sigma, St. Louis, MO) were used to prepare 20 g/100 mL stock solutions. The calibration set ($n = 60$) was composed of solutions containing the tertiary mixtures of sugars at concentration levels of 0, 2, 4, and 8 g/100 mL. The ranges were chosen to evaluate the adequacy of the method for application to fruit juice samples.[18] The prediction capabilities of the PLSR-NIR model were evaluated on commercial apple (four brands) and orange (two brands) juices that were purchased from a local store. Different lots of the commercial juice samples were analyzed in triplicate. The excess pulp material in suspension in orange juices (20 mL) was removed by collecting the supernatant after centrifugation of the samples on a Sorvall RC-5B refrigerated superspeed centrifuge (DuPont Instruments, Wilmington, DE) that operated at 7719g for 20 min. The solutions (0.4 mL) that were analyzed by diffuse reflectance were deposited on 37 mm diameter glass microfibre filter

disks (Whatman GF/C, UK) placed inside a 40 mm diameter petri dish base (Perkin–Elmer, Norwalk, CT). The samples were vacuum dried for 20 min in a laboratory oven (National Appliances Co., Portland, OR) that operated at temperatures between 35 and 40 °C.

FT-NIR measurements.—All FT-NIR spectra were recorded using a Perkin–Elmer Spectrum Identicheck system operating at 8 cm^{-1} resolution. The mirror velocity was 0.30 cm/s. Diffuse reflectance and transflectance measurements were made by using a diffuse reflection integrating sphere, equipped with a PbS detector. Solutions (3 mL) were dispensed in a 40 mm diameter petri dish base (Perkin–Elmer) placed on the reflectance accessory for direct measurement by transflection using an aluminum diffuse reflector (Perkin–Elmer). The reflector contained integral spacers that allow two passes of the beam through the sample to provide a total pathlength of 0.5 mm. In the case of the reflectance measurements, the petri dish base containing the filter disks with the dried sample was placed onto the reflectance accessory. Transmittance measurements were made using a 0.5 mm glass cell (Perkin–Elmer), and a deuterated triglycine sulfate (DTGS) detector. The absorbance spectrum was obtained by ratioing the single beam spectrum against that of the background: 'spectralon' (a teflon-based material from LabSphere, North Sutton, NH) for reflectance and transflectance; or air for transmittance. The FT-NIR spectra were recorded from 10,000 to 4000 cm^{-1} at intervals of 2 cm^{-1}. The interferograms (64) were co-added followed by strong Beer–Norton apodization. The total number of data points was 3001 for each spectrum. Prior to calibration, the FT-NIR reflectance data were mean centered, smoothed with a 25-point polynomial-fit Savitzky–Golay function and further transformed with standard normal variate (SNV) and detrending pre-treatment to correct multiplicative interferences, variation in baseline shift and curvilinearity.[19] The processed spectral data were transformed by a Savitzky–Golay second derivative.

Multivariate analyses.—PLSR was applied to generate calibration models using a QUANT PLUS (Perkin–Elmer) software system. The optimum number of latent variables (LV) used for prediction was determined by full cross-validation (leave-one-out). The model producing the minimum standard error of prediction (SEP) was selected as the best model for the spectral data set. The resulting models were evaluated in terms of loading vectors, standard error of estimate (SEE), the standard error of cross validation (SECV), coefficient of determination (R^2), and F-value for the calibration models. The SEE gives an indication of the quality-of-fit of the regression, and is calculated as the square root of the residual variance divided by the number of degrees of freedom. The SECV is an estimate of the standard error of prediction (magnitude of error expected when independent samples are predicted using the model). The coefficient of determination gives the proportion of variability of the property that is described by the model. The F-value can be viewed as a measure of the signal-to-noise in the model as it determines whether the property variance is significantly better than the residual property variance. If the value of F is low, the calibration is not robust and the performance on future unknown samples is likely to be substantially worse than on calibration samples. If F is large, then the calibration should have approximately the same accuracy on future unknown samples as on the calibration samples.

The X-residuals and leverage were used for the evaluation of outliers. Calibration models were evaluated graphically to ensure a random distribution of residuals. An observation exhibiting large residuals or an unusual residual pattern normally indicates an outlier. The leverage of a calibration sample was used to determine its potential contribution to the estimated calibration model. Any observation with abnormal residual or leverage was reanalyzed and eliminated if necessary after which the calibration model was recalculated.

Recovery studies.—To perform recovery studies, commercially available samples of juice (apple and orange) were spiked with stock solutions containing levels of 0.025, 0.05 and 0.1 g/mL of sucrose, glucose, and fructose. Three stock solutions were prepared us-

ing different combinations of the sugar concentrations described above (Solution A: 0.05 g/mL of sucrose, 0.1 g/mL glucose, and 0.1 g/mL fructose; Solution B: 0.1 g/mL of sucrose, 0.025 g/mL glucose, and 0.05 g/mL fructose; and Solution C: 0.025 g/mL of sucrose, 0.05 g/mL glucose, and 0.025 g/mL fructose). Five independent samples (vials) of juice (9 mL/vial) were spiked with 1 mL of each stock solution. The control was independent samples of juice (five) spiked with 1 mL of deionized distilled water. Each measurement was performed in triplicate. The percentage recovery was determined from the relative differences between the control and added values, multiplied by 100.

Enzymatic assays for sugars.—Juices (1:100 dilution) were analyzed by using enzymatic kits for glucose, fructose and sucrose (Sigma). The method is based on the oxidation of 6-phosphogluconate in the presence of nicotinamide adenine dinucleotide (NAD) in a reaction catalyzed by glucose-6-phosphate dehydrogenase. During this oxidation, an equimolar amount of NAD is reduced to NADH resulting in an increase in absorbance at 340 nm that is directly proportional to the sugar concentration. The absorbance was measured on a Beckman DU640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA). Quantitation of sugars was done by a standard curve for sucrose, glucose, and fructose at concentrations of 5–50 ppm.

HPLC analysis.—Quantitation of sugars was done by a standard curve containing sucrose, glucose, and fructose at concentrations of 2.5, 5, 7.5, and 10 ppm. The juices were diluted to 1:4000 ratio with deionized distilled water in order to obtain detection responses within the range of the standard curve.

Sugars were separated using an analytical Dionex high-performance liquid chromatograph (LC) equipped with a pulsed amperometric detector with a gold working electrode and the data was collected and analyzed by Dionex PEAKNET Workstation software. Column: CarboPac PA1 analytical (5 micron), 4 × 250 mm ID (Dionex Co., Sunnyvale, CA) with a guard column (4 × 50mm). Mobile phase: 150 mM NaOH prepared from a 50% (w/w) NaOH solution that contains low carbonate. The LC system was run at a flow rate of 1 mL/min and an injection volume of 10 μL was used. Solvents and samples were filtered through a 0.45 μm Millipore filter type HA (Millipore Corp., Bedford, MA). The electrode was maintained at the following potentials and durations: $E_1 = 0.1$ V ($T_1 = 400$ ms), $E_2 = -2.0$ V ($T_2 = 10$ ms), rapid excursion to 0.6 V (E_3) and $E_4 = -0.1$ V ($T_4 = 60$ ms). The integration time was set for 200–400 ms at E_1.[20]

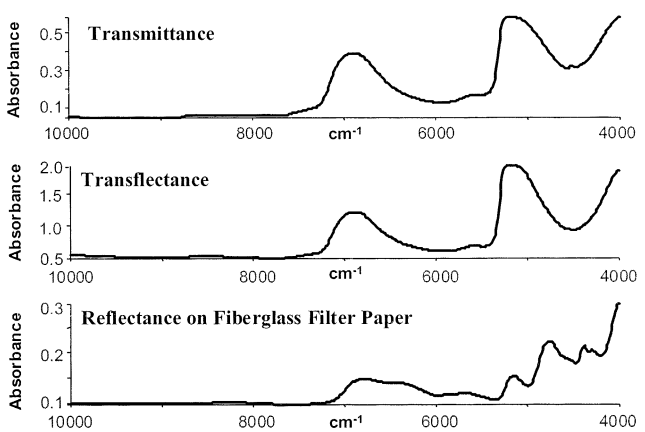

Fig. 1. Characteristic FT-NIR absorbance spectra of sugar solutions using different sampling devices.

3. Results and discussion

Development of the PLS calibration model for sugars in aqueous systems.—The FT-NIR spectra of sugar solutions obtained by transmittance, transflectance and reflectance are presented in Fig. 1. The strong water absorption peaks centered at 6900 cm^{-1} (first O–H overtone) and 5200 cm^{-1} (O–H combination) overlapped the analyte spectral signal for samples measured by transmittance and transflectance techniques. Elimination of the solvent and analysis of the dried sugar extract on glass microfibre paper by diffuse reflectance allowed the extraction of several spectral features, similar to those reported in Ref. 12 for sugar solutions.

Mathematical pre-treatments of the NIR data were used to enhance the prediction ability of the models and the qualitative interpretation of the spectra. The standardization of the spectra by using smoothing and SNV preprocessing steps improved the signal-to-noise

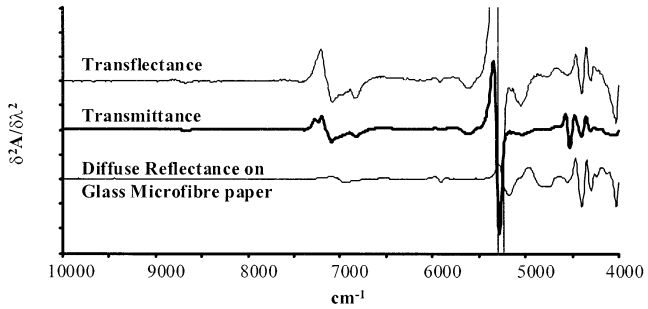

Fig. 2. Second derivative (37 window size) spectra of sugar solutions using different sampling devices.

ratio of the data and corrected for the nonlinear light scattering effects of the samples (i.e., suspended particles, bubbles) and baseline offsets.[19] The Savitzky–Golay second derivative transform allowed the extraction of useful band information (Fig. 2) through the removal of baseline variations and resolution of overlapping peaks.[21,22] Furthermore, the strong effect of water FT-NIR signals were minimized by using the derivatized spectra on transmittance measurements with a 0.5 mm cell cuvette (Fig. 2).

The cross-validated (leave-one-out) PLSR model results are shown in Table 1. The optimal derivatized spectral data had a window size of 37 points (Table 1). The larger second derivative window size suggests that the most important spectral features being modeled were broad and that the data contained interfering variance (noise) that was attenuated by using more smoothing. The offset preprocessed (underivatized) and derivatized spectra (data not shown) had similar performance statistics, however, in most cases the second derivative transformation of the spectral data (Table 1) had 6–18% lower standard error of cross validation, lowered the number of latent variables, and gave 20–50% higher *F*-values (model robustness) compared to the PLSR analysis with untransformed data.

The PLSR models generated from diffuse reflectance measurements of dried sugar extracts embedded on glass microfibre supports showed lower performance statistics, with SECV values three and ten times higher than values for models obtained by transflectance and transmittance derivatized spectra, respectively. Our data contrast with the improved standard errors of prediction of sugar concentration in solutions which have been reported using glass microfibre supports with estimated prediction errors of 0.183% for sucrose, 0.355% for glucose and 0.301% for fructose[12] and 0.302% for sucrose, 0.157% for glucose and 0.154% for fructose.[11] These researchers

Table 1
Multivariate analysis by partial least-squares regression [a] of sugar solutions

Contaminant	No. of latent variables	SEE	SECV	Multiple correlation	% variance (R^2)	*F*-value
A. Transmittance						
Selected regions 7500–7000; 6000–5400; 4700–4000 cm^{-1}						
Sucrose	6	0.069	0.078	1.00	99.94	12,950
Glucose	5	0.086	0.095	1.00	99.90	9,980
Fructose	5	0.038	0.044	1.00	99.98	52,200
B. Transflectance						
Selected regions 7300–6700; 6000–5400; 4800–4150 cm^{-1}						
Sucrose	5	0.171	0.228	1.00	99.60	2439
Glucose	5	0.157	0.214	1.00	99.68	2967
Fructose	5	0.134	0.178	1.00	99.80	4780
C. Diffuse reflectance on glass microfibre paper						
Selected regions 10,000–4000 cm^{-1}						
Sucrose	3	0.501	0.546	0.98	96.48	420
Glucose	2	0.704	0.762	0.97	93.45	335
Fructose	3	0.585	0.632	0.98	95.68	340

The spectral data were transformed by using second derivative (37 gap size).

[a] Cross-validated model performances ($n = 60$) for FT-NIR data that were pre-processed by a 25 point smooth function and standard normal variate (SNV) with de-trending transformation.

SEE: standard error of estimate; SEP: standard error of prediction.

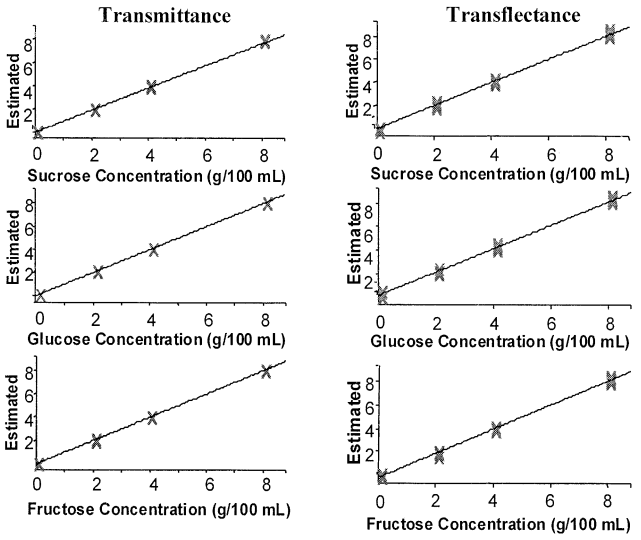

Fig. 3. Cross-validated (leave-one-out) partial least-squares regression plots for sugar solutions calibration set.

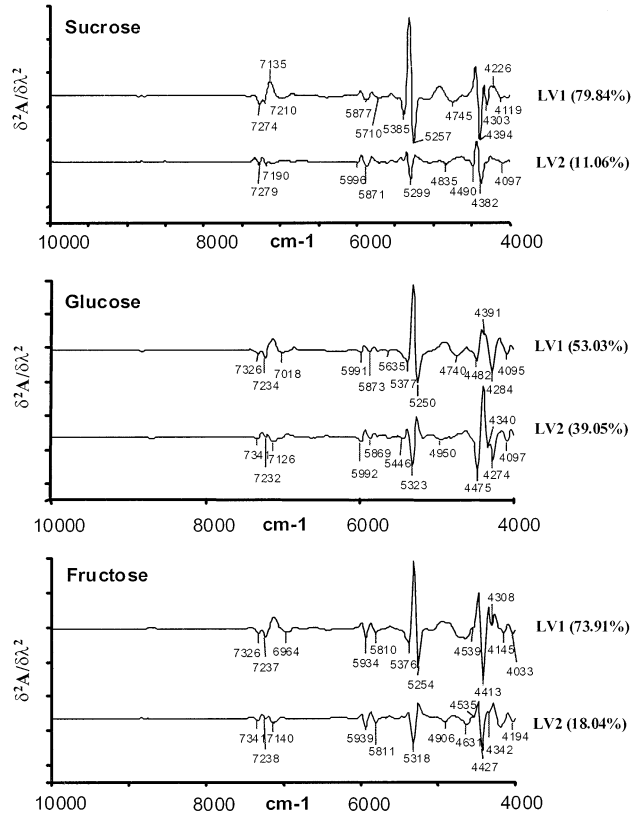

Fig. 4. Partial least-squares loading spectra for the first two latent variables for the calibration set measured by transmittance and transformed by second derivative (37 window size).

used a special DESIR (Dry Extract System for Infrared) unit to obtain a homogeneous dry-extract after rapid solvent elimination. In the present study, a conventional vacuum oven was used to remove the solvent that may have caused a sugar concentration gradient in the filters, thus resulting in our lower performance statistics. Nevertheless, Segtnan and Isaksson[23] reported that direct transmittance measurements in cuvettes gave better prediction results than DESIR measurements.

Models generated from transmittance spectra gave the best performance with standard error of prediction (SECV) $< 0.10\%$, R^2 of 99.9%, and F-value > 6000. The FT-NIR transmittance technique allowed the development of a single PLSR model for direct measurement of sugar solutions with remarkably lower prediction errors compared to those reported for similar aqueous systems.[10,12,24]

The PLS regression graphs (Fig. 3) for sugar solutions measured by transmittance and transflection showed good correlation between the specified sugar levels and the FT-NIR estimated concentrations. Most of the variance (Fig. 4) was explained by the first two latent variables ($> 91\%$). Examination of these loading spectra indicated which areas of the spectrum were associated with the highest variation in the calibration set. Frequencies of high variation reflect contributions of spectral resolution elements that can be correlated with combinations of different chemical and physical phenomena.[15,25] The PLSR loading spectra (Fig. 4) extracted important absorption features for sucrose, glucose and fructose in solution with absorption bands due to O–H and C–H groups in the carbohydrates region largely influencing the spectral variation. The loading spectra showed that the highest variation in the calibration set was associated with frequencies in the 7500–6700 cm^{-1} and 6000–4000 cm^{-1} region. Absorption bands at 7130 cm^{-1} and 7340–7200 cm^{-1} were related to frequencies of first overtones of O–H stretching modes[13] and C–H combination vibrations,[5] respectively. Bands in the 5950–5700 cm^{-1} could be assigned to first overtones of C–H stretching modes.[13] The sharp absorption band near 5250 cm^{-1} has been related to second overtones of O–H stretch/C–H stretch modes.[26] The information-rich region from 4600 to 4000 cm^{-1} can be ascribed to combinations of O–H bend/hydrogen-bonded O–H stretch (~ 4428 cm^{-1}), O–H stretch/C–C stretch (~ 4393 cm^{-1}) and combinations of

C–H/C–C (4385–4063 cm^{-1}) vibrations of the sugar molecules.[26]

Comparison of PLSR-FT-NIR models generated by the spectroscopic sampling devices versus enzymatic and HPAE-PAD methods.— To determine the applicability of the proposed PLSR-FT-NIR method based on standard sugar solutions to the analysis of sugars in commercial samples, a set of apple and orange juices were evaluated. The dry-extract FT-NIR reflectance technique using glass microfibre filters was not used because of the high-standard error of prediction observed on a limited number of apple juice samples. In addition, it was a more time-consuming method and after the drying procedure, some samples showed light yellow–brown spots (browning reaction) that affected the FT-NIR reproducibility.

The sugar concentration predicted by using PLSR-FT-NIR models was compared to that measured by high-performance anion-exchange chromatography using pulsed amperometric detector (HPAE-PAD), a precise and accurate method for sugar analysis.[20] PLSR-FT-NIR calibration models using spectra that were transformed to their second-derivative (37 point window) and using selected spectral ranges (7500–7000; 6000–5400; 4700–4000 cm^{-1}) gave the closest predicted values for sugar content in the juices to those obtained by the HPAE-PAD reference method (Table 2). The use of calibration models with offset pre-processing gave inaccurate predictions of sugar concentrations in all juices compared to the reference method, even though it gave very good performance statistics. It appears that other components of the juices contain spectral information that cannot be discriminated by the model generated from non-derivatized spectra, resulting in imprecision in the prediction of the sugar concentration. The multiple analysis of variance (ANOVA) showed significant differences ($p < 0.01$) in sugar content of the juices depending on the type of methodology used (HPAE-PAD, FT-NIR transmittance and FT-NIR transflectance). Multiple-range tests (least significant difference, LSD) were used to determine which means were significantly different from each other. In the case of sucrose analysis, the multiple range tests revealed that the three methodologies were significantly different from each other ($p < 0.0001$). The PLSR-FT-NIR methods (transmittance and transflectance) over-predicted the sucrose content in apple juices, except for brand D where the PLSR-FT-NIR transmittance model predicted the sucrose values very close to those obtained by HPAE-PAD. However, the multiple range tests showed that for the determination of glucose and fructose there was no significant difference in the precision of the PLSR-FT-NIR transmittance and HPAE-PAD methods for the apple and orange juice matrices. The PLSR-FT-NIR transflectance methodology gave comparable predictions to those of the HPAE-PAD reference method only for glucose in orange juice and fructose in apple juice. The multiple ANOVA also showed a significant effect of the brand of apple or orange juice ($p < 0.001$) on the sugar (sucrose, glucose and fructose) content.

The ability of the PLSR-FT-NIR models to predict the sugar content in apple juices was also compared to enzymatic methods for sucrose, glucose and fructose. The multiple ANOVA showed that there were no significant differences ($p > 0.05$) in the determination of sucrose by enzymatic and PLSR-FT-NIR transmittance methods. However, the glucose and fructose content estimated by the PLSR-FT-NIR models were higher than those estimated by enzymatic assays (Table 2). The glucose and fructose levels predicted by the PLSR-FT-NIR models were very similar in both experiments (HPAE-PAD and enzymatic methods), even though different lots of the apple juice samples were analyzed.

The sugar concentration of commercial apple and orange juices predicted by the PLSR-FT-NIR models were similar to average values reported in the literature. Brause and Raterman[27] reported that apple juices should contain 1–3% sucrose, 2–3% glucose and 5–8% fructose. Elkins et al.[28] reported mean values ($n = 92$) of 1.66% sucrose, 2.42% glucose and 6.07% fructose in apple juices by HPLC. Dillon[18] reported typical sugar ranges in orange juices of 3–5.5% sucrose, 1.8–2.8% glucose, and 1.8–2.8% fructose, while Li et

Table 2
Comparison [a] of high performance anion-exchange (HPAE) chromatography with pulsed amperometric detector (PAD) and enzymatic assay kits to FT-NIR spectroscopic methods for the determination of sugars in fruit juices

Sample	Sucrose (g/100 mL)	Glucose (g/100 mL)	Fructose (g/100 mL)	F/G ratio	Sucrose (g/100 mL)	Glucose (g/100 mL)	Fructose (g/100 mL)	F/G ratio	Sucrose (g/100 mL)	Glucose (g/100 mL)	Fructose (g/100 mL)	F/G ratio
	HPAE-PAD				FT-NIR transmittance spectroscopy				FT-NIR transflectance spectroscopy			
Apple juice												
A	1.53 (0.05)	2.78 (0.03)	6.56 (0.11)	2.4	2.27 (0.03)	2.66 (0.03)	6.72 (0.03)	2.7	1.93 (0.10)	2.58 (0.07)	6.99 (0.06)	2.7
B	1.21 (0.04)	2.91 (0.07)	6.38 (0.26)	2.2	1.96 (0.03)	3.02 (0.04)	6.51 (0.03)	2.2	1.59 (0.19)	3.27 (0.11)	6.60 (0.22)	2.0
C	1.05 (0.02)	3.18 (0.04)	6.51 (0.11)	2.0	1.75 (0.05)	3.34 (0.04)	6.38 (0.04)	2.0	1.32 (0.10)	3.65 (0.26)	6.53 (0.08)	1.8
D	1.06 (0.05)	3.13 (0.11)	7.96 (0.30)	2.5	1.11 (0.04)	2.95 (0.04)	7.77 (0.07)	2.7	0.87 (0.21)	3.15 (0.12)	7.83 (0.14)	2.5
Orange juice												
E	4.88 (0.35)	2.23 (0.12)	2.54 (0.17)	1.1	4.61 (0.05)	2.20 (0.06)	2.99 (0.03)	1.4	4.48 (0.25)	2.06 (0.05)	3.16 (0.15)	1.5
F	4.06 (0.16)	2.63 (0.15)	3.12 (0.22)	1.2	3.85 (0.05)	2.94 (0.17)	2.98 (0.03)	1.0	3.54 (0.25)	3.09 (0.11)	3.09 (0.08)	1.0
	Enzymatic assay kit				FT-NIR transmittance spectroscopy				FT-NIR transflectance spectroscopy			
B	1.86 (0.46)	2.09 (0.05)	6.17 (0.50)	3.0	1.86 (0.06)	3.01 (0.05)	6.64 (0.01)	2.3	2.15 (0.10)	2.99 (0.20)	6.27 (0.14)	2.1
D	1.72 (0.43)	2.39 (0.03)	6.24 (0.53)	2.6	1.64 (0.02)	3.03 (0.06)	7.46 (0.02)	2.6	1.94 (0.13)	3.00 (0.15)	7.08 (0.08)	2.4

[a] The values presented are means of four independent juice samples from the same lot. The standard deviations are in parenthesis.

al.[11] reported mean values ($n = 218$) of 3.73% sucrose, 2.75% glucose and 2.06% fructose in orange juice by enzymatic assays.

The sugar recoveries for the analysis of spiked fruit juices (Table 3) were $>93\%$ for apple and orange using the PLSR-FT-NIR transmittance method while the PLSR-FT-NIR transflectance method showed more variation with recovery ranging from 76 to 146%.

The PLSR-FT-NIR models, especially using the transmittance mode, can reproducibly and precisely predict the glucose and fructose content in apple and orange juices. Replicated FT-NIR transflectance measurements showed that the technique produced outliers that required re-evaluation of the samples. Therefore, duplicate or triplicate readings from each sample were necessary to obtain reliable data, which resulted in larger data sets and longer analysis times when compared to the transmittance technique.

Nevertheless, the PLS-FT-NIR models overestimated the levels of sucrose in apple juices as compared to HPAE-PAD method, the sucrose values obtained were similar to those reported by Elkins[28] and the results from the enzymatic assay. Furthermore, the predicted sucrose levels were very reproducible as shown by the low standard deviations (Table 2) of the FT-NIR measurements. The sucrose deviations from the ion chromatographic values could be due to the presence of interfering compounds (i.e. organic acids) and the use of ion-exchange resins could improve the sucrose predictive ability of PLS-FT-NIR models. Changes in environmental temperature could also affect the FT-NIR spectra.[29]

Evaluation of sugar content of fruit juices.— Direct measurements of the juice samples by using the PLSR-FT-NIR transmittance model showed good reproducibility with low standard deviations between replicated measurements of apple and orange juices (Table 4) from the same lot. Furthermore, the same lot of apple juice for brands B and C was independently analyzed 1 week apart and very comparable sugar levels were obtained (Table 4, first two rows). Several lots of the same commercial juice brands were evaluated and some variability in the mean sugar levels can be observed among samples. Factors such as variety, region, maturity, processing practices,

Table 3
Sugar recoveries [a] (%) of spiked apple and orange juices using partial least-squares regression (PLSR)-FT-NIR models

Sample	Analyte	Added sugar (g)	Concentration recovered by transmittance (g)	Recovery (%)	Concentration recovered by transflectance (g)	Recovery (%)
Apple juice	Sucrose	0.025	0.024 (0.002)	95	0.021 (0.002)	82
		0.050	0.052 (0.004)	103	0.052 (0.005)	103
		0.100	0.098 (0.003)	98	0.104 (0.025)	104
	Glucose	0.025	0.027 (0.006)	109	0.024 (0.001)	94
		0.050	0.048 (0.006)	96	0.038 (0.001)	76
		0.100	0.097 (0.005)	97	0.085 (0.011)	85
	Fructose	0.025	0.025 (0.001)	99	0.025 (0.001)	98
		0.050	0.046 (0.003)	93	0.049 (0.011)	98
		0.100	0.096 (0.002)	96	0.098 (0.006)	98
Orange juice	Sucrose	0.025	0.026 (0.002)	104	0.037 (0.001)	146
		0.050	0.056 (0.005)	112	0.051 (0.013)	102
		0.100	0.102 (0.006)	102	0.100 (0.009)	100
	Glucose	0.025	0.027 (0.003)	108	0.031 (0.010)	124
		0.050	0.051 (0.007)	101	0.038 (0.014)	76
		0.100	0.103 (0.08)	95	0.087 (0.014)	87
	Fructose	0.025	0.023 (0.002)	102	0.023 (0.003)	92
		0.050	0.048 (0.005)	103	0.044 (0.007)	88
		0.100	0.096 (0.002)	96	0.105 (0.016)	105

[a] The values presented are means of triplicate recovery experiments. Each replicate consisted of five independent juice samples that were spiked at three different levels of sugars. The standard deviations are in parenthesis.

Table 4
Prediction of sugar concentration by using partial least-squares regression (PLSR)-FT-NIR transmittance calibration models for apple and orange juice analysis

Sample [a]	Sucrose (g/100 mL)	Glucose (g/100 mL)	Fructose (g/100 mL)	F/G ratio
Apple juices [b]				
A	1.62 (0.03)	3.26 (0.02)	6.57 (0.04)	2.0
$n=3$	1.56 (0.07)	3.19 (0.14)	6.65 (0.05)	2.1
	2.18 (0.05)	2.70 (0.05)	6.98 (0.06)	2.6
	2.27 (0.01)	2.53 (0.02)	6.74 (0.01)	2.7
B	1.92 (0.03)	3.17 (0.08)	6.72 (0.04)	2.1
$N=4$	1.86 (0.06)	3.18 (0.06)	6.62 (0.07)	2.1
	1.58 (0.05)	3.22 (0.08)	6.44 (0.04)	2.0
	1.96 (0.02)	2.93 (0.05)	6.54 (0.02)	2.2
	1.90 (0.05)	2.94 (0.06)	6.64 (0.02)	2.3
C	2.30 (0.06)	2.75 (0.08)	6.76 (0.04)	2.5
$n=3$	2.31 (0.05)	2.64 (0.07)	6.76 (0.09)	2.6
	2.74 (0.08)	2.45 (0.08)	6.61 (0.04)	2.7
	1.75 (0.04)	3.26 (0.02)	6.42 (0.04)	2.0
D	1.65 (0.03)	2.87 (0.02)	7.51 (0.07)	2.6
$n=4$	1.58 (0.04)	3.16 (0.04)	7.47 (0.02)	2.4
	1.11 (0.04)	2.85 (0.05)	7.79 (0.07)	2.7
	1.68 (0.02)	2.87 (0.05)	7.46 (0.02)	2.6
Orange juice				
E	4.44 (0.06)	2.61 (0.04)	2.99 (0.02)	1.2
$n=5$	4.50 (0.03)	2.16 (0.05)	3.11 (0.03)	1.4
	4.59 (0.06)	2.14 (0.04)	3.03 (0.03)	1.4
	4.43 (0.04)	2.25 (0.03)	3.05 (0.03)	1.4
F	4.00 (0.07)	3.02 (0.10)	2.81 (0.03)	0.9
$n=5$	4.04 (0.05)	2.96 (0.04)	2.83 (0.06)	1.0
	3.89 (0.02)	2.92 (0.06)	2.90 (0.02)	1.0
	3.87 (0.06)	2.89 (0.11)	2.98 (0.03)	1.0
	3.82 (0.02)	2.98 (0.04)	2.87 (0.02)	1.0

[a] The letters represent different commercial juices purchased from a local store. Each row represents mean values of different sample lots that were analyzed within 1 week of purchase by 3–5 independent replicates ($n=$ number of juice cartons). The standard deviations are in parenthesis.

[b] The same lot of apple juice for brands B (first two rows) and C (first two rows) were independently analyzed 1 week apart.

and storage, among others, can affect the sugar composition of the juices. It was noted that for brand C, the apple juice of the first three sample lots was made from juice concentrate from Chile and Argentina while the fourth sample lot was made from concentrate from USA, South Africa and Italy. Evaluation of apple juice samples that were stored at room temperature in their sealed containers for 9 months (e.g., juice brand B had mean values of $1.22 \pm 0.06\%$ sucrose, $3.41 \pm 0.10\%$ sucrose and $7.14 \pm 0.07\%$ fructose and brand D had a mean value of $0.66 \pm 0.02\%$ sucrose, $3.98 \pm 0.10\%$ sucrose and $7.95 \pm 0.09\%$ fructose) versus those measured within a week of purchase (Table 4) showed that FT-NIR transmittance technique could detect an overall decrease in sucrose and an increase in glucose and fructose, probably due to an inversion reaction. The sensitivity of the PLSR-FT-NIR transmittance method could be applied for monitoring microbial spoilage/contamination of fruit juices due to the fermentation of sugars which results in changes in sugar profile. The sugar content changed considerably in apple juice samples that developed mold spoilage that is, juice A (third row) showed mean values of 0.85% sucrose, 3.35% glucose, and 7.59% fructose after evidence of mold contamination; similarly, juice D (first row) showed mean values of 0.13% sucrose, 3.58% glucose, and 8.41% fructose after substantial mold contamination.

The rapid sugar analysis of apple juices by the PLSR-FT-NIR models could be used to monitor authenticity by using a fructose to glucose (F/G) ratio of 1.6 minimum and a sucrose maximum of 3.5%.[27] The calculated F/G ratios of the apple juices evaluated (Tables 2 and 4) ranged from 2.0 to 3.0. The PLSR-FT-NIR technique could also be a valuable tool in quality control for the rapid determination of juices with long storage times or unusual heat treatments. The implementation of on-line NIR sensors or devices for the continuous monitoring of the sugar content of fruit juices could allow better product standardization. The current method would be appropriate for on-line NIR sensors for clear juices such as apple juice and turbid juices such as no/low pulp orange juices. The effect of scattering light due to suspended particles (i.e., pulp) on the performance of the model would need to be evaluated to expand the technology.

4. Conclusions

The application of FT-NIR spectroscopy and PLSR multivariate techniques allowed for the simultaneous quantitation of individual sugars in juices. The PLSR-FT-NIR models generated from transmittance spectra reproducibly and precisely predicted the individual sugar content in different juice matrices, including clarified apple juice (scatter-free) and orange juice (which gives some scattering properties) using a simple external calibration prepared with sugar standard solutions. FT-NIR spectroscopy allowed for the rapid, accurate and non-destructive analysis of sugars in juices and could be applied in quality control of beverages or to monitor for adulteration and contamination. Furthermore, this technique allows the quantification of glucose, fructose and sucrose simultaneously in juice solutions to make carbohydrate analysis in fruit juices more amenable to routine measurements.

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