A Combined Approach of Infrared Spectroscopy and Multivariate Analysis for the Simultaneous Determination of Sugars and Fructans in Strawberry Juices During Storage

Lucía Cassani, Mauricio Santos, Esteban Gerbino, María del Rosario Moreira, and Andrea Gómez-Zavaglia

Abstract: In this work, a Fourier transform mid-infrared spectroscopy (FTIR)-based method was developed for simultaneously quantifying simple sugars and exogenously added fructooligosaccharides (FOS) in strawberry juices preserved for up to 14 d using nonthermal techniques (geraniol and vanillin+ultrasound). The main spectral differences were observed in the 1200 to 900 cm^{-1} region. The presence of FOS was identified by the typical bands at 1134, 1034, and 935 cm^{-1}. During storage, a significant decrease of sucrose was concomitant to an increase of glucose and fructose in juices stored without any previous preservation treatment, as determined by high-performance liquid chromatography (HPLC). A principal component analysis was performed on the FTIR spectra corresponding to the different treatments. The groups observed explained more than 94% of the variance and were related to changes in the carbohydrate composition during storage. Then, different partial least square models (PLS) were defined to determine the concentrations of glucose, sucrose, fructose, and those of exogenously added FOS with degrees of polymerization within 3 and 5. The carbohydrates' concentrations determined by HPLC were used as reference method. The models were validated with independent sets of data. The mean of predicted values fitted nicely those obtained by HPLC (correlation and $R^2 > 0.97$), thus supporting the use of the PLS models to monitor the quality of strawberry juices in unknown samples. In conclusion, FTIR spectroscopy appears as an adequate analytical tool to quick assess whether juice formulations meet specifications in terms of authenticity, contamination and/or deterioration.

Keywords: carbohydrates, chemometrics, fruit-based product, FTIR spectroscopy, functional ingredients

Practical Application: FTIR spectroscopy provided a method potentially transferable to the food industry when associated with the multivariate analysis. The robust 21 PLS models defined in this work provided reliable tools for the rapid monitoring of juices' authenticity and/or deterioration. In this regard, FTIR associated to multivariate analysis enabled the determination of different sugars in a single measurement without the need of pure sugars as standards. This experimental simplicity supports the use of FTIR at the production line, and also contributes to save time in determining carbohydrates' composition and stability, in an environmentally friendly way.

Introduction

The consumption of nonthermally preserved fruit juices has increased in the last decades in response to consumer's demand for fresh, healthier, safe, and ready-to-eat foods. Strawberry juice is one of the most consumed juices because it has an excellent acceptability and is considered a rich source of antioxidant compounds (Oszmiański and Wojdyło 2009). To attend the demand of healthier food, food products can be enriched with functional ingredients, such as fructooligosaccharides (FOS) and inulin, thus increasing their nutritional properties (Keenan and others 2011).

Simple sugars are the main soluble components in strawberry, with glucose, fructose, and sucrose accounting for almost 99% of total sugar content. These compounds are considered as important quality indicators by both consumers and food industry (Kallio and others 2000). The product quality is a major issue of competition among producers. In this regard, the stability of soluble sugars in fruits or fruit juices (that is, glucose, fructose, sucrose) and that of those eventually added as nutritional ingredients (FOS) are important parameters for the food industry, as they are directly related to the quality and authenticity of fruit-based products (Fügel and others 2005). For this reason, determining variations in the sugar content of fresh fruits or in their juices is an important indicator of adulteration, contamination (Rodriguez-Saona and others 2001), and even deterioration during storage (Jacxsens and others 2003), which requires quick and reliable analyses.

In general, the concentrations of sugars in food products (particularly in juices and beverages) are estimated using refractive index measurements or volumetric procedures, which provide

JFDS-2017-1365 Submitted 8/18/2017, Accepted 10/30/2017. *Authors Cassani and Moreira are with Research Group of Food Engineering, Faculty of Engineering, National Univ. of Mar del Plata, Buenos Aires, Argentina. Author Cassani is also with Argentinean Agency for the Scientific and Technological Promotion (ANPCyT), Argentina. Authors Gerbino, Moreira, and Gómez-Zavaglia are with Argentinean National Research Council (CONICET), Buenos Aires, Argentina. Authors Gerbino and Gómez-Zavaglia are also with Center for Research and Development in Food Cryotechnology (CIDCA, CCT-CONICET La Plata) RA1900, La Plata, Argentina. Authors Santos is with Clinical Bacteriology Service, Department of Bacteriology, National Institute for Infectious Diseases (ANLIS-INEI), Dr Carlos G. Malbran, Argentina. Direct inquiries to author, Lucía Cassani, (E-mail: lcassani@fi.mdp.edu.ar)*

doi: 10.1111/1750-3841.13994

Journal of Food Science A Publication of the Institute of Food Technologists

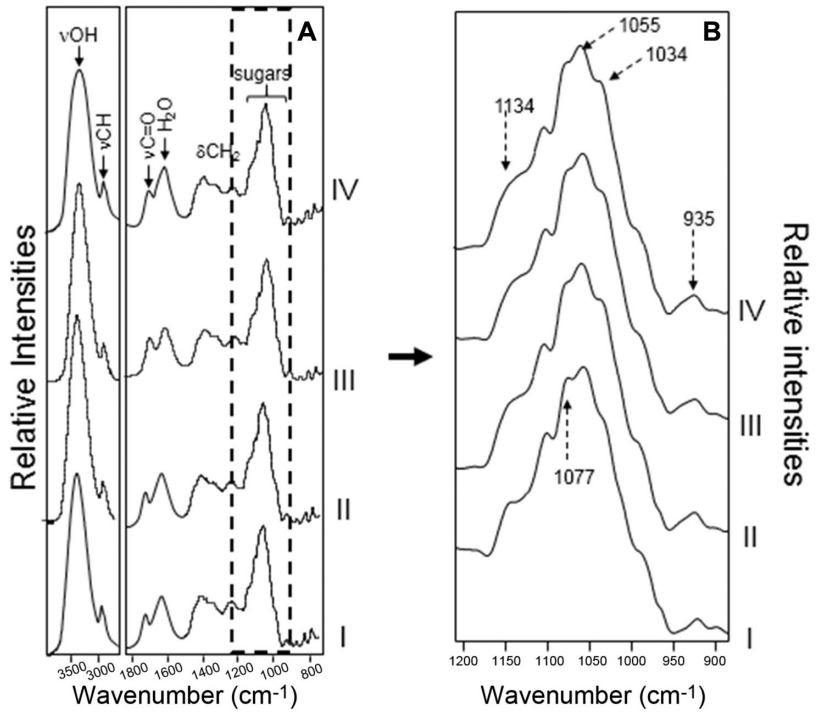

Figure 1–FTIR spectra of freshly prepared strawberry juices: (A) whole spectra; (B) 1200 to 900 cm^{-1} region.
I. Juice with no preservation treatments
II. Juice enriched with inulin/oligofructose and no preservation treatments
III. Juice enriched with inulin/oligofructose and treated with geraniol
IV. Juice enriched with inulin/oligofructose and treated with vanillin + ultrasound

information about the total sugar content and the amount of reducing sugars (glucose and fructose), respectively. For separately quantifying each sugar, several methods can be employed, including enzymatic analysis and chromatographic methods (Duarte and others 2002). Trends in analytical chemistry are towards simple, quick and if possible, nondestructive methods. Fourier transform infrared spectroscopy (FTIR) has demonstrated to be a reliable analytical technique with some advantages over traditional methods, namely rapidity, cost effectiveness, simplicity, nondestructive, almost no preparation of samples and no need of exogenous chemical reagents. Besides, the analytical assays do not generate hazardous wastes and can be used for routine analysis if proper calibrations and validations are developed (Caramês and others 2016; Hirri and others 2016). For these reasons, FTIR has been used to determine the concentration of different sugars in chemically complex media (Kwak and others 2007; Leopold and others 2011; Blanch and others 2012). When combined with multivariate analysis, it provides an efficient tool for the expeditious determination of sugars in different fruit juices (Leopold and others 2011), cellulose, or pectin (Fellah and others 2009), among other food-related products.

FTIR and multivariate analysis have also been used to correlate the sugar composition of apricots and mangos with their ripeness state (Duarte and others 2002; Bureau and others 2009), to classify citrus juices (Hirri and others 2015), or to determine adulteration in pomegranates (Vardin and others 2008), apple, orange, and peach juices (Sivakesava and others 2001; Leopold and others 2011) and the composition of apple pomaces (Queji and others 2010; Gabriel and others 2013). In strawberries, FTIR has been employed to discriminate fruits and leaves from different cultivars (Kim and others 2009) or to determine the thermophysical properties of FOS during postharvest storage (Blanch and others 2012). In spite of the efforts made in determining the sugar composition of different fruit products using FTIR, up to our knowledge no attempts have been made to monitor the carbohydrate composition of juices during storage hereto, which is an important indicator of microbial deterioration and therefore, a quality parameter. For this reason, the goal of this work was to use a combined approach of FTIR and multivariate analysis to quantitatively determine the composition of simple sugars and FOS, as quality and nutritional parameters of fiber-enriched strawberry juices during storage (2 wk at 5 °C). To reach this goal, FTIR spectra were registered at regular intervals during storage, and then different partial least squares (PLSs) models were defined to determine the concentrations of sucrose, glucose, fructose, and FOS with degrees of polymerization (DP) within 3 and 5, using high-performance liquid chromatography (HPLC) results as reference method.

Materials and Methods

Strawberry juice preparation

Strawberries (*Fragaria x ananassa* Duch.) were grown and harvested at commercial maturity in Sierra de los Padres (Mar del Plata, Argentina). The fruits were washed with tap water and processed with a juicer (Moulinex, Buenos Aires, Argentina). The liquid obtained was homogenized and divided into 4 portions, which were bottled into 350 mL polyethylene terephthalate flasks under hygienic conditions and sealed with polyethylene caps to be subsequently formulated.

Juice formulation

Four different beverages were prepared. One of them was simply strawberry juice without any addition or preservation treatment (control). The 2nd juice sample was enriched with 15 g/L of inulin/oligofructose mixture (5:3 ratio, Grupo Saporiti, Argentina) but not submitted to any preservation treatment (enriched control). The 3rd juice was also enriched with inulin/oligofructose, as described before, and then treated with geraniol (0.225 μL/mL),

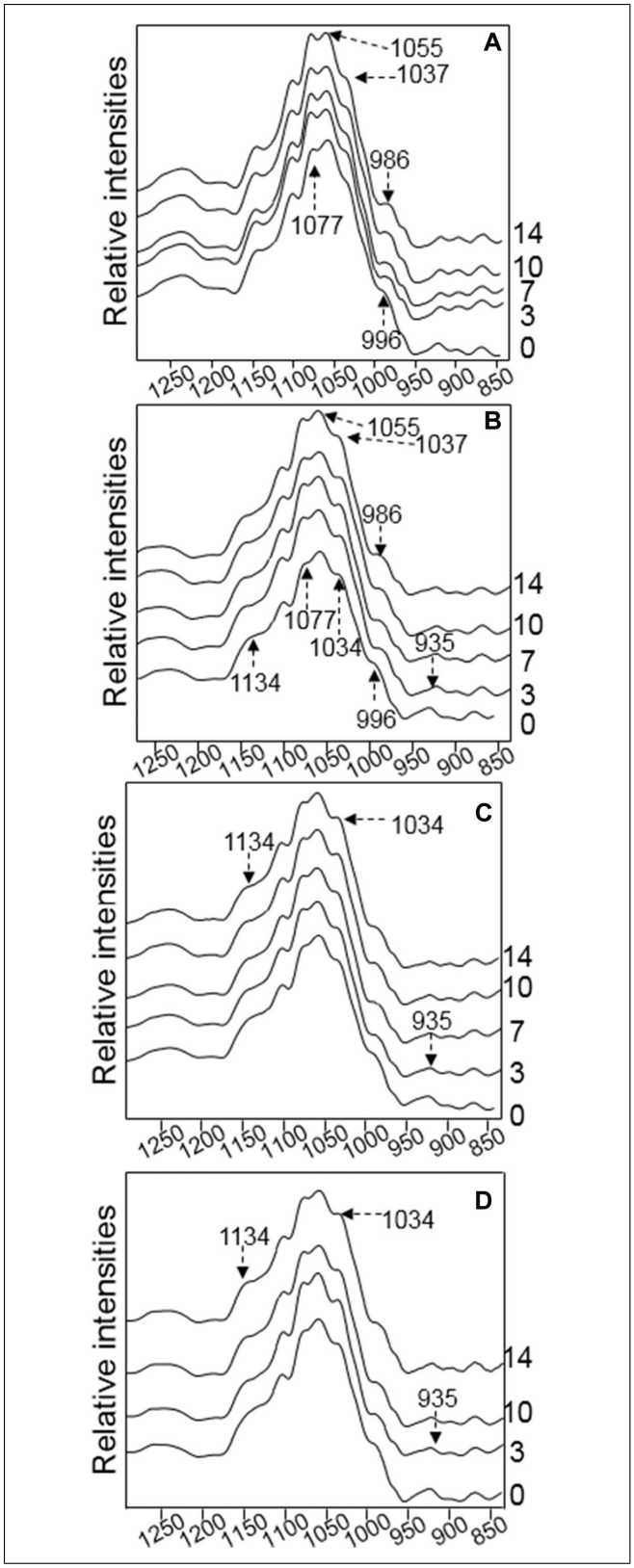

Figure 2–FTIR spectra of strawberry juices after 0, 3, 7, 10, and 14 d of storage at 5 °C in the 1200 to 900 cm^{-1} region: (A) juice with no preservation treatments; (B) juice enriched with inulin/oligofructose and no preservation treatments; (C) juice enriched with inulin/oligofructose and treated with geraniol; (D) juice enriched with inulin/oligofructose and treated with vanillin + ultrasound. Numbers on the right side of each plot indicate days of storage.

according to Cassani and others (2016). The last juice was enriched with inulin/oligofructose and then, treated with a vanillin combined with ultrasound preservation treatment. Vanillin (1.25 mg/mL) was added to the fiber-enriched juice, then immersed in a water bath of an ultrasonic chamber (15 × 29 × 15 cm; TestLab, Argentina) at 20 °C for 7.5 min (sonication conditions: frequency 40 kHz, power 180 W). The inulin/oligofructose ratio, vanillin concentration, and ultrasound conditions were established using response surface methodology, as reported in Cassani and others (2017a).

In summary, 4 different samples were used for the subsequent experiments.

(i) Juice with no preservation treatment
(ii) Juice enriched with inulin/oligofructose with no preservation treatment
(iii) Juice enriched with inulin/oligofructose and treated with geraniol
(iv) Juice enriched with inulin/oligofructose and treated with vanillin + ultrasound

After treatments, all the 4 beverages were stored at 5 °C for 0, 3, 7, 10, and 14 d in the dark. At each storage time, 100 mL of juice were collected, frozen for 48 h at −80 °C and freeze-dried for 48 h at −50 °C using a Mod FD-1A-50 equipment (Boyikang, China). Freeze-dried samples were stored at −20 °C until FTIR and HPLC analyses. Three replications (3 juice bottle per storage day) were performed and the experiment was conducted twice.

HPLC analysis

The carbohydrate composition of the juices was determined by an HPLC Spectra SYSTEM Isocratic Pump P100 with refractive index detector and a Rheodyne injection valve with a 20 μL-sample loop (Sigma-Aldrich, Miss., U.S.A.). The chromatographic column was a Sugar Pak I column (10 μm, 6.5 mm × 300 mm) for carbohydrate analysis (Milford, Mass., U.S.A.). Column temperature was maintained at 80 °C. The freeze-dried samples were diluted, filtered through 0.22 μm Millipore Durapore membranes (Billerica, Mass., U.S.A.) and eluted with milli-Q water (mobile phase) at a flow-rate of 0.5 mL/min. Chromatograms were integrated using WinPCcrom XY 2.0 software (Buenos Aires, Argentina).

Standards of fructose, glucose, sucrose, 1-kestose (DP3), nystose (DP4), and 1^F-fructofuranosyl nystose (DP5) were used to determine their retention times and check the linear range of the measurements. The concentration of each sugar in the juices was determined using calibration curves performed with the above-mentioned standards.

FTIR

In parallel to HPLC assays, FTIR spectra were registered on the freeze-dried juices. The spectra were registered in the 4000 to 500 cm^{-1} range on KBr pellets, prepared with a ratio of 1 mg sample/200 mg KBr. For each sample, at least 10 FTIR spectra were registered. Spectra were recorded in a transmission mode by co-adding 64 scans with 4 cm^{-1} spectral resolution, using OMNIC software (version 8.3, Thermo Scientific, Mass., U.S.A.) on a Thermo Nicolet iS10 spectrometer (Thermo Scientific).

Multivariate analysis

Multivariate analysis and data preprocessing (mean centering correction) were performed on the FTIR spectra registered in the

Table 1–Predicted carbohydrates' composition (mg/g dry matter) of different juice samples as determined by FTIR after applying partial least square models.

		Time of storage (d)			
Analyte	**0**	**3**	**7**	**10**	**14**
		Untreated juice (group A)			
Sucrose	35.29 ± 0.12^{a}	4.29 ± 0.13^{b}	4.13 ± 0.10^{b}	4.23 ± 0.15^{b}	4.08 ± 0.12^{b}
Glucose	185.08 ± 0.92^{b}	235.30 ± 2.19^{a}	250.15 ± 2.57^{a}	248.44 ± 1.42^{a}	55.42 ± 1.49^{c}
Fructose	192.49 ± 0.79^{b}	252.88 ± 2.25^{a}	251.54 ± 2.88^{a}	263.02 ± 1.48^{a}	67.35 ± 1.34^{c}
		Juice + inulin/oligofructose (group B)			
Sucrose	57.06 ± 0.30^{a}	21.46 ± 0.21^{b}	14.60 ± 0.37^{b}	20.45 ± 0.17^{b}	20.35 ± 0.13^{b}
Glucose	178.59 ± 0.84^{c}	246.36 ± 1.43^{a}	144.98 ± 3.43^{d}	213.10 ± 0.55^{b}	240.12 ± 1.93^{a}
Fructose	196.74 ± 0.82^{c}	269.01 ± 1.40^{a}	142.07 ± 3.50^{d}	236.04 ± 0.62^{b}	260.84 ± 1.41^{a}
DP3	4.26 ± 0.01^{a}	4.09 ± 0.00^{a}	2.97 ± 0.03^{b}	2.98 ± 0.01^{b}	3.59 ± 0.01^{ab}
DP4	3.45 ± 0.00^{a}	3.01 ± 0.01^{a}	1.97 ± 0.05^{b}	3.04 ± 0.02^{a}	3.26 ± 0.03^{a}
DP5	2.55 ± 0.01^{c}	3.78 ± 0.01^{b}	5.47 ± 0.09^{a}	2.29 ± 0.06^{c}	2.19 ± 0.04^{c}
		Juice + inulin/oligofructose + geraniol (group C)			
Sucrose	60.00 ± 0.15^{a}	19.62 ± 0.30^{b}	16.63 ± 0.22^{b}	21.24 ± 0.22^{b}	17.91 ± 0.34^{b}
Glucose	188.37 ± 0.41^{ab}	200.17 ± 0.08^{a}	194.01 ± 0.15^{a}	152.25 ± 0.21^{c}	170.82 ± 0.75^{b}
Fructose	200.37 ± 0.43^{ab}	220.49 ± 0.14^{a}	214.58 ± 0.16^{a}	168.34 ± 0.21^{c}	191.80 ± 0.86^{b}
DP3	4.30 ± 0.01^{a}	3.45 ± 0.01^{b}	3.36 ± 0.02^{b}	3.02 ± 0.01^{b}	3.31 ± 0.01^{b}
DP4	3.43 ± 0.01^{b}	4.01 ± 0.00^{a}	3.64 ± 0.00^{b}	2.42 ± 0.01^{c}	3.39 ± 0.02^{b}
DP5	3.17 ± 0.00^{b}	4.18 ± 0.01^{a}	4.20 ± 0.01^{a}	4.26 ± 0.01^{a}	4.80 ± 0.01^{a}
		Juice + inulin/oligofructose + vanillin + ultrasound (group D)			
Sucrose	48.96 ± 0.41^{a}	20.81 ± 0.21^{b}	12.54 ± 0.21^{c}	24.23 ± 0.36^{b}	10.36 ± 0.25^{c}
Glucose	127.45 ± 0.23^{b}	132.46 ± 0.59^{b}	133.94 ± 0.29^{b}	208.38 ± 1.95^{a}	137.76 ± 2.31^{b}
Fructose	142.61 ± 0.64^{b}	148.71 ± 0.12^{b}	130.50 ± 0.72^{bc}	221.65 ± 0.85^{a}	145.62 ± 1.35^{b}
DP3	3.52 ± 0.01^{b}	4.41 ± 0.01^{a}	3.51 ± 0.01^{b}	4.26 ± 0.02^{a}	2.63 ± 0.03^{c}
DP4	2.43 ± 0.00^{b}	3.18 ± 0.01^{ab}	2.50 ± 0.00^{b}	3.64 ± 0.01^{a}	1.87 ± 0.03^{c}
DP5	4.37 ± 0.01^{a}	2.65 ± 0.00^{b}	2.55 ± 0.01^{b}	2.84 ± 0.00^{b}	3.63 ± 0.02^{ab}

Data are shown as mean ± standard deviation of 5 determinations. Values with different letters in the same row indicate significant differences ($P < 0.05$) during storage time. DP3, 1-kestose; DP4, nystose; DP5, 1^{F}-fructofuranosyl nystose.

previous section, using The Unscrambler® software (version 10.2, CAMO, Norway).

Principal component analyses (PCAs) were performed on the FTIR spectra corresponding to juices exposed to the 4 different treatments, after 0, 3, 7, 10, and 14 d of storage.

Then, different PLS models were defined for each group of juices (21 PLS as a whole) to determine the concentrations of glucose, sucrose, fructose, and those of exogenously added FOS with DP within 3 and 5. To this aim, groups of 25 FTIR spectra covering the whole range of sugars' concentrations, including spectra obtained from independent experiments and repetitions, were used. The carbohydrates' concentrations obtained by HPLC were used as reference method. The reliability and robustness of the calibrated models were determined as function of their coefficient of determination, R-square, BIAS, and their calibration and prediction errors (RMSEC and RMSEP, indicating the average difference between predicted and experimentally obtained results). High correlation values and low errors indicate a good capacity to correctly predict or classify unknown samples. A set of 25 spectra independently registered, and corresponding to different preparations from those used for calibration were used to validate the models. All the information regarding the set up the PLS models is shown in Table S1.

Statistical analysis

A completely randomized design was used for each experiment. Results reported in this work are mean values accompanied by their standard errors. Experimental data were analyzed using *R*, software version 2.12 (R Development Core Team, 2011). Analysis of variance ($P < 0.05$) was performed and Tukey–Kramer comparison test was used to estimate significant differences through storage time ($P < 0.05$).

Results and Discussion

Structural analysis of carbohydrates by FTIR spectra

Figure 1(A) shows the FTIR spectra of the 4 groups of freshly prepared juices. A general pattern of bands was observed in all spectra in the 4000 to 1200 cm^{-1} region. The strong band at 3389 cm^{-1} corresponds to the νOH vibrational mode, those at 2935 and 2891 cm^{-1}, to the νCH vibrational modes, the band at 1724 cm^{-1}, to the νC = O vibrational mode of esters or organic acids (that is, methyl galacturonates of pectins) and that at 1634 cm^{-1}, to water molecules embedded in the juice matrix (Coimbra and others 1998). The bands at around 1400 cm^{-1} arose from the δCH_2 vibrational modes. Because the spectra of juices preserved using nonthermal techniques were similar to those untreated, it can be concluded that the preservation treatments employed (geraniol and vanillin combined with ultrasound) did not lead to structural changes in the freshly prepared juices. This is an expected result, since the preservation treatments do improve the quality parameters of strawberry juices without damaging their molecular structure (Cassani and others 2017b).

The 1200 to 900 cm^{-1} region deserves a special comment. This region, also known as "fingerprint region" of sugars (Santos and others 2014), showed noticeable differences among groups of juices (Figure 1B). The main vibrational modes absorbing in this region include the δC–O–C of the glycosidic linkage, the δCOH, and the νC–C. Even when it is difficult to assign the vibrational modes corresponding to each individual band, the bands in this region collectively provide a complex pattern that doubtless

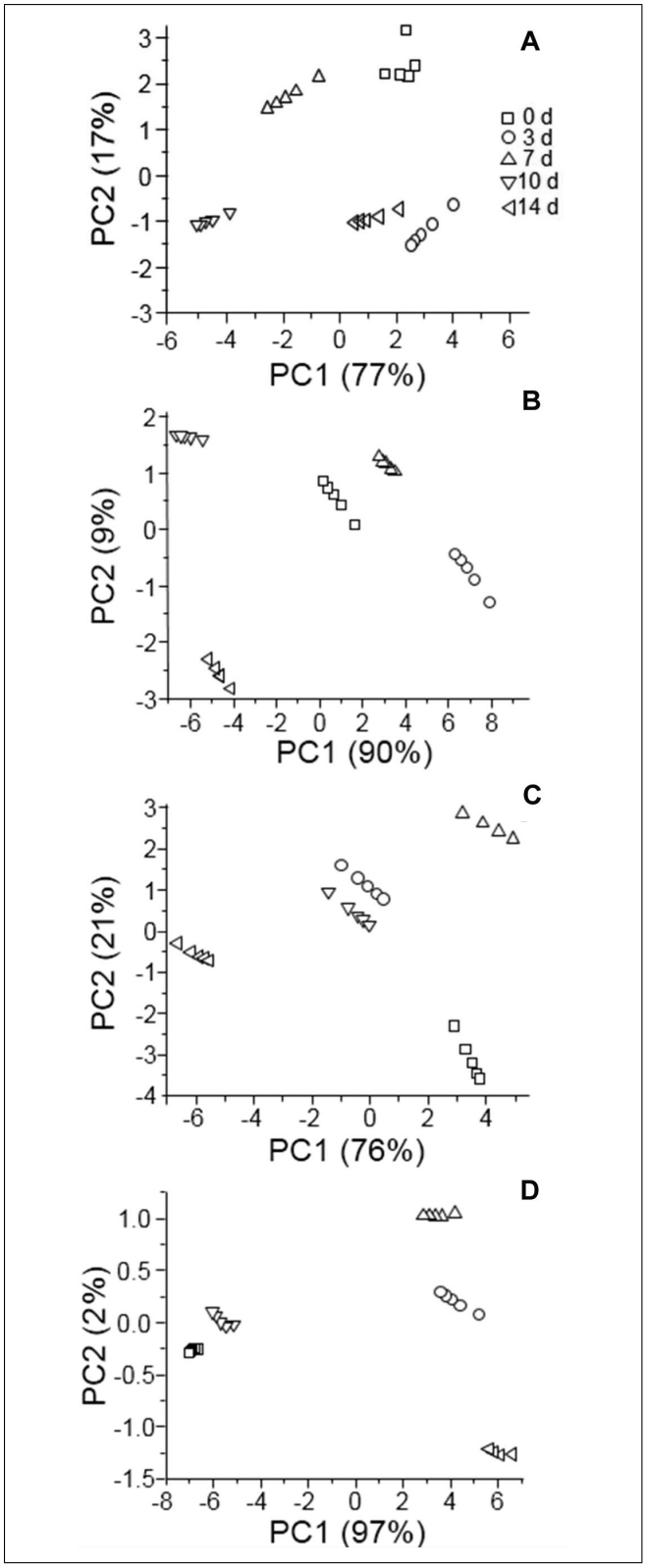

Figure 3–PC2 versus PC1 performed on strawberry juices after 0, 3, 7, 10, and 14 d of storage at 5 °C. (A) Juice with no preservation treatments; (B) juice enriched with inulin/oligofructose and no preservation treatments; (C) juice enriched with inulin/oligofructose and treated with geraniol; (D) juice enriched with inulin/oligofructose and treated with vanillin + ultrasound. Different symbols indicate different times of storage: squares (0), circles (3 d), up triangles (7 d), down triangles (10 d), left-oriented triangle (14 d).

characterizes a given compound and thus, can be used to identify pure sugars. A mixture of different mono and disaccharides together with FOS and inulin coexist in the strawberry juices investigated in this work. This leads to complex spectra of largely overlapped bands resulting from the contribution of all the carbohydrates present in the juices. The noticeable changes observed in the fingerprint region were related to both the composition of the freshly prepared juices (Figure 1B) and the time of storage (changes within each group during storage; Figure 2).

When compared the freshly prepared strawberry juices enriched with inulin and oligofructose with those juices without any fiber addition (control), some features deserve to be described (Figure 1B). The presence of FOS in the juices can be identified by the intense bands at 1134, 1034, and 935 cm^{-1}, as previously reported by Romano and others (2016). The band at 1055 cm^{-1} is present in all groups and is ascribed to sucrose (Leopold and others 2011). In juices prepared without fiber addition (control), the band at 1077 cm^{-1} (reported as characteristic of fructose) was more intense relatively to that at 1055 cm^{-1}. On the contrary, among fiber enriched juices, the band ascribed to fructose weakened regarding that of sucrose. This indicates that the fructose/sucrose ratio for freshly prepared juices without the addition of exogenous fiber was higher than that of fiber-enriched samples, which is consistent with the results presented in Table 1 for time equal 0.

The differences observed within each group of samples during storage indicate changes in the sugar composition of strawberry juices (Figure 2). The juices prepared without addition of preservative compounds (Figure 2A and B) were those showing the main spectral changes during storage. The main spectral differences observed for the 4 groups of juices during 14 d of storage at 5 °C are briefly explained below:

(i) In juices stored without any preservation treatment (Figure 2A and B), the band at 1077 cm^{-1} (fructose) increased at expenses of that at 1055 cm^{-1} (sucrose; Leopold and others 2011). Similar results were found by Romano and others (2016), who observed a typical band of sucrose at 1054 cm^{-1}. The increase of fructose at expenses of sucrose indicates the hydrolysis of this latter sugar, and is consistent with the yeast and molds' metabolism (the main spoilage flora in strawberry; Cassani and others 2017b).

(ii) The very weak shoulder occurring at 1037 cm^{-1} at time equal to 0 in juices without any treatment (Figure 2A and B), became a clear shoulder after 14 d of storage. It can be ascribed to an increase of glucose (Leopold and others 2011; Romano and others 2016). As it was mentioned before, the increase in glucose concentration is attributed to the hydrolysis of sucrose (Table 1).

(iii) The shoulder at 996 cm^{-1} (typical of sucrose) shifted to a more intense and defined shoulder occurring at 986 cm^{-1} during storage (Figure 2A and B), reported as characteristic of fructose (Leopold and others 2011). This latter shoulder is also consistent with the production of fructose as result of the consumption of sucrose (Table 1) by spoilage flora (Cassani and others 2017b).

(iv) Among fiber-enriched samples (Figure 2B–D), a noticeable band at 1134 cm^{-1}, typical of FOS was observed. No decrease of intensity was observed for this band during storage. In addition, a shoulder at 1034 cm^{-1} and a band a 935 cm^{-1}, also ascribed to FOS (Romano and others 2016), was observed in all fiber-enriched juices and did not change its intensity during

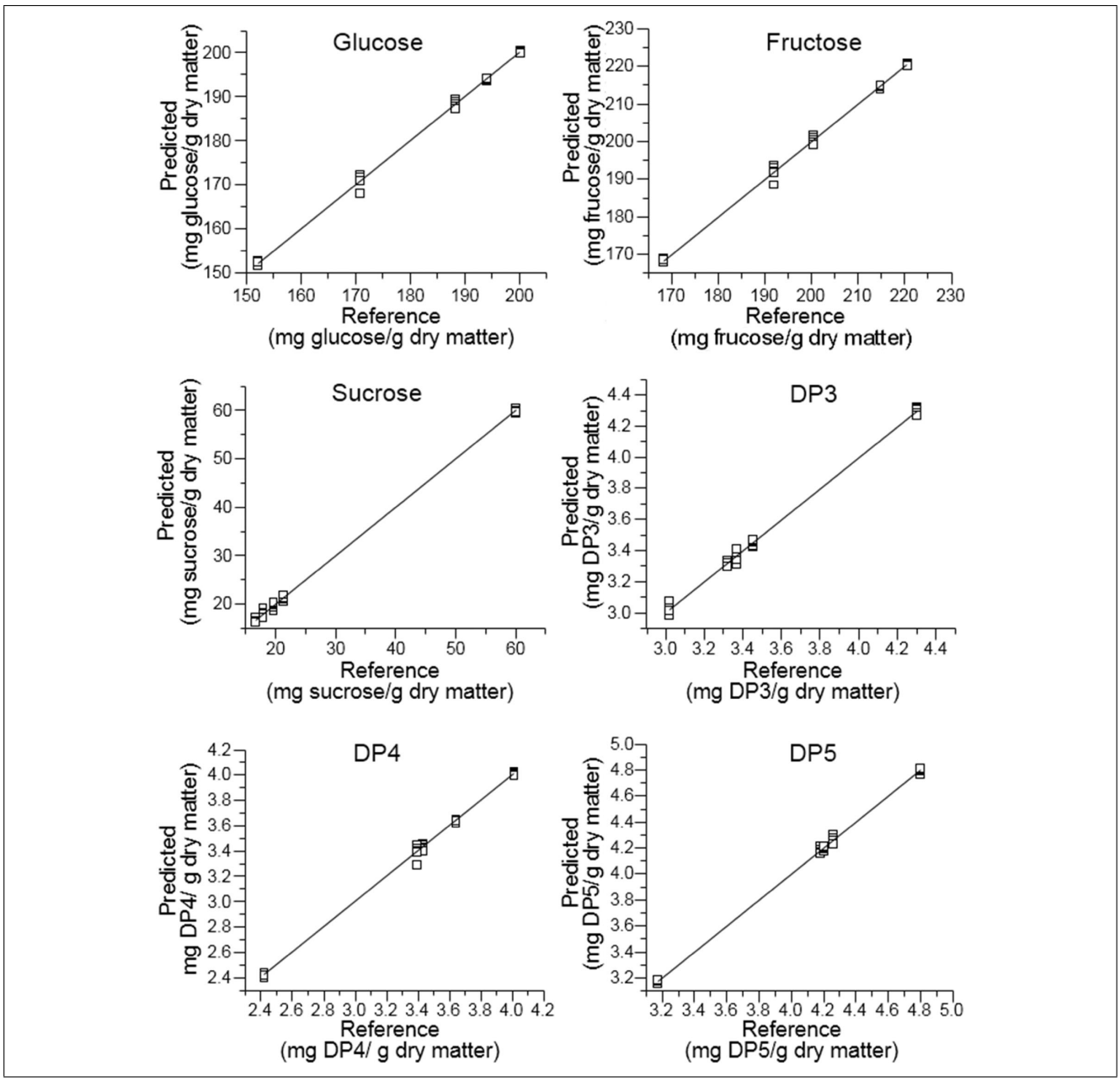

Figure 4–Predicted versus reference concentrations of glucose, fructose, sucrose, DP3, DP4, and DP5 for strawberry juice enriched with inulin/oligofructose and treated with geraniol.

storage. This shows the stability of FOS in all fiber-enriched juices.

PCA and PLS regression

Even when a thorough observation of the FTIR spectra provides clear information about qualitative changes in the sugar composition of juices during storage, the large amount of data present in the FTIR spectra might include certain information that can be disregarded if no deeper analysis is performed. In particular, the doubtless identification of FOS of different DP or the quantification of simple sugars during storage cannot be investigated from the raw spectra and require the use of multivariate analysis.

For this reason, a PCA was carried out on the 4 groups of juices along the storage time (Fig 3). The PC1 and PC2-loading plots corresponding to each group of juices are shown in Fig S1. In consonance with Figure 1 and 2, PC1 was the PC explaining the greatest differences among samples (76% to 97%). These differences were mainly in the 1200 to 900 cm^{-1} region for all the 4 treatments considered. In turn, as the percentage of variance explained by PC2 was only within 2% to 21%, no clear relation with structural differences could be explained on this PC. The scores plots performed on the 4 types of juices showed 5 different groups, corresponding to the 5 different times of storage (0, 3, 7, 10, and 14; Figure 3A–D). In all cases, PC1 explained a greater percentage of variance than PC2, and as a whole PC1 and PC2 explained more than 94% of the total variance. Juice samples preserved using nonthermal techniques (Figure 3C and D), at day 0 of storage were placed in opposite direction to those at day 7 and 14, indicating that the carbohydrate composition, especially sucrose and FOS content, changed during storage. This is in agreement

with the results shown in Table 1. Similarly, those juices without preservation treatments (Figure 3A and B), at day 7 of storage, were placed in opposite direction to those at day 14, which clearly indicates differences in their sugars content. This is ascribed to changes in the monosaccharides content during storage which is also consistent with results obtained in Table 1.

In a further step, we aimed at quantifying the sugar composition of both freshly prepared juices and juices stored for up to 14 d at 5 °C. To this aim, different PLS models were defined for each group of juices, using results obtained by HPLC as the reference method. This information enabled the definition of 6 different PLS models to quantify sucrose, glucose, fructose, DP3, DP4, and DP5 in those juices that were enriched with fructans (nonthermally treated samples and enriched control), and 3 different PLS models to quantify the 1st 3 simple sugars in juices prepared with no addition of FOS (control). This approach led to the definition of 21 different PLS models for the quantification of sugars.

All PLS models were calibrated using 25 spectra and validated with an independent set of data composed of 25 spectra, collected under the same conditions (Table S1). Figure 4 depicts the independent validation results for the prediction models developed to determine the studied carbohydrates in fiber-enriched strawberry juices treated with geraniol. The results for the prediction models developed for the rest of the samples are shown in Figure S2 to S4. The statistical parameters showed the robustness of all the PLS models defined, with a low RMSEC and high correlation and R^2 values (>0.97). The complete data set of the performance calibration and validation processes are shown in Table S2 to S5. The mean of predicted values fitted nicely the results obtained by HPLC and are shown in Table 1. As a whole, the obtained results support the predictive capacity of the PLS models to investigate unknown samples.

Conclusions

This study provided vibrational spectroscopy information about strawberry juices enriched with functional ingredients and preserved for 2 wk using nonthermal techniques. FTIR spectroscopy allowed not only a fundamental study but also provided a method potentially transferable to the food industry when associated with the multivariate analysis.

The thorough investigation of the FTIR spectra enabled the comprehension of the chemical reactions involved in juices' deterioration (that is, hydrolysis of sucrose), which was consistent with changes in the PLS predicted values of carbohydrates during storage. From an applied point of view, the stability of sugars during storage of juices is an important parameter to determine their deterioration. In addition, the concentration of the different sugars in freshly prepared juices is an indicator of authenticity and/or contamination that may be related with their adulteration. These 2 issues represent a major concern among juice producers, for whom quick analytical methods, as those developed in this work, can provide an important contribution. The robust 21 PLS models defined in this work provided reliable tools for the rapid monitoring of juices' authenticity and/or deterioration. In this regard, it is worth to mention that FTIR associated to multivariate analysis enabled the determination of different sugars in a single measurement without the need of pure sugars as standards. This experimental simplicity supports the use of FTIR at the production line, also contributes to saving time in determining carbohydrates' composition and stability, in an environmentally friendly way. This way, quality control analyses could be performed directly by registering FTIR spectra, in a much simpler way than the traditional methods currently used (that is, HPLC).

In summary, the results obtained support the use of FTIR spectroscopy as an adequate analytical tool to quick assess whether strawberry juice formulations meet specifications in terms of authenticity, contamination, and/or deterioration.

Acknowledgments

This work was supported by the Argentinean Agency for the Scientific and Technological Promotion (ANPCyT; Projects PICT/2014/0912, PICT/2012/1121 and PICT/2016/4808), Argentinean Research Council (CONICET), and the National Univ. of Mar del Plata.

Author's Contributions

L.C. and E.G. did the experimental work. M.S. did the multivariate analysis. L.C. and A.G.-Z. coordinated the work (conception of the work, analysis, and discussion of results). L.C., M.R.M., and A.G.-Z. wrote the manuscript. All authors have approved the final version of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Data set preparation and performance results of partial least squares (PLS) analyses for sucrose, fructose, glucose, DP3, DP4, and DP5 for each group of juices.

Table S2. Statistical values of the calibration and validation sets for the PLS models corresponding to sucrose, fructose, and glucose in strawberry juice without any addition (control).

Table S3. Statistical values of the calibration and validation sets for the PLS models corresponding to sucrose, glucose, fructose, DP3, DP4, and DP5 in strawberry juice enriched with inulin/oligofructose with no preservation treatment (enriched control).

Table S4. Statistical values of the calibration and validation sets for the PLS models corresponding to sucrose, glucose, fructose, DP3, DP4, and DP5 in strawberry juice enriched with inulin/oligofructose, further treated with geraniol.

Table S5. Statistical values of the calibration and validation sets for the PLS models corresponding to sucrose, glucose, fructose, DP3, DP4, and DP5 in strawberry juice enriched with inulin/oligofructose, further treated with vanillin and ultrasound.

Figure S1. 1-D Loading plots in PC1 (A) and PC2 (B) performed on the FTIR spectra of Figure 1.

Figure S2. Predicted vs. reference values of strawberry juices with no preservation treatment.

Figure S3. Predicted vs. reference values of strawberry juices enriched with inulin/oligofructose with no preservation treatment.

Figure S4. Predicted vs. reference values of strawberry juices enriched with inulin/oligofructose and treated with vanillin and ultrasound.