

ARTICLES

Heterospectral Two-Dimensional Correlation Spectroscopy of Mid-Infrared and Fourier Self-Deconvolved Near-Infrared Spectra of Sugar Solutions

ROBERT A. COCCIARDI, ASHRAF A. ISMAIL,* YAN WANG, AND
 JACQUELINE SEDMAN

Department of Food Science and Agricultural Chemistry, McGill University, 21111 Lakeshore Road,
 Ste-Anne de Bellevue, Québec, Canada H9X 2V9

The mid- and near-infrared (mid-IR and NIR) spectra of aqueous solutions of glucose and fructose, fructose and galactose, and glucose and galactose were recorded and analyzed by heterospectral two-dimensional correlation spectroscopy (H2D-CS) to determine characteristic NIR wavelengths for each sugar. Fourier self-deconvolution (FSD) was applied to the NIR spectra prior to H2D-CS analysis to help resolve the strongly overlapping sugar absorptions. Examination of the H2D-CS data gave characteristic absorption wavelengths for glucose, fructose, and galactose. The wavelengths identified by H2D-CS were then used to develop multiple linear regression (MLR) calibrations for the quantitative analysis of mixtures of the three sugars in solution. This approach gave comparable results to MLR calibrations based on wavelengths selected by examination of the first- and second-derivative spectra of solutions of the individual sugars.

KEYWORDS: Heterospectral two-dimensional correlation spectroscopy (H2D-CS); sugar solutions; near-infrared (NIR); mid-infrared (mid-IR); Fourier self-deconvolution (FSD)

INTRODUCTION

Near-infrared (NIR) spectroscopy is a widely used instrumental method for the compositional analysis of food products largely due to its speed and capability to determine multiple components simultaneously without the need for solvents or reagents (1, 2). Although mid-infrared (mid-IR) spectroscopy shares many of these advantages, NIR spectroscopy has a substantial advantage in terms of ease of sample handling because it allows for longer pathlengths and the use of glass/quartz cells or optical fibers for process control applications (2). From the standpoint of calibration development, however, mid-IR spectroscopy has the advantage that distinctive bands can be associated with different functional groups and constituents of interest, whereas NIR spectra are difficult to interpret, consisting largely of broad bands that are the superposition of various combinations and overtones of the fundamental vibrational modes that give rise to mid-IR absorption bands. Furthermore, in complex matrices, multiple components interact, making the NIR spectrum very complicated. As a result, quantitative analysis by NIR spectroscopy has relied heavily on the use of chemometric techniques to relate subtle spectral

variations to changes in concentration of a particular component in the sample matrix, resulting in the need for a large number of calibration standards to construct a calibration model. Calibration development is generally a time-consuming, iterative process as the selection of wavelengths or spectral regions to be used for calibration is generally done empirically (3).

The drawbacks described above may be exemplified by NIR analysis of sugars in solutions, such as beverages, where the low absorptivity of sugars and the strong overlap of individual sugar absorptions as well as their extensive overlap with water absorptions make calibration particularly difficult (4, 5). Although the analysis of sugars in fruit juices and botrytized-grape sweet wines by NIR spectroscopy has been successfully carried out (6–9), the further development of quantitative NIR spectroscopic methods for sugar analysis would benefit from a better understanding of the NIR spectra of sugars and more precise band assignments.

In 1993, Robert et al. employed canonical correlation analysis to study carbohydrate absorptions in the mid-IR and NIR spectral regions and to identify NIR wavelengths characteristic to individual carbohydrates (10). Also in 1993, Noda introduced generalized two-dimensional (2D) correlation spectroscopy, a technique capable of extracting information not available from a one-dimensional (1D) spectrum (11). In the 2D correlation

* Author to whom correspondence should be addressed [telephone (514) 398-7991; fax (514) 398-7977; e-mail ashraf.ismail@mcgill.ca].

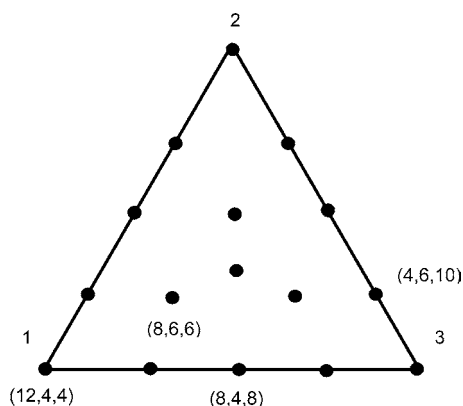


Figure 1. Optimal mixture design for a three-component system.

approach, the spectral data are spread over a second dimension, simplifying the visualization of complex overlapping bands and enhancing spectral resolution (12). Over the past decade, a wide range of applications of this technique have been reported, including heterospectral 2D correlation (H2D-CS) studies, whereby the data from two different spectroscopic methods are correlated (13). H2D-CS has been used for band assignments and spectral interpretation of polymers (14–17), proteins (18–20), forages (21), lignins (22), rice (23), hard red winter and spring wheats (24), sugar and lysozyme mixtures (25), glucose anomers (26), and sugar beets (27). H2D-CS studies are especially powerful in unraveling NIR spectral information by correlating it to well-defined bands in other types of spectra.

In the present study, H2D-CS was applied for the first time to mid-IR and NIR spectra of mixtures of sugars (glucose, fructose, and galactose) with the objective of identifying specific wavelengths in the NIR region that are characteristic of each individual sugar in such mixtures. In view of previous work demonstrating the utility of spectral resolution enhancement by Fourier self-deconvolution (FSD) in improving the definition of features on 2D contour maps (28), H2D-CS analysis was carried out on the FSD rather than on the raw FTNIR spectra. The wavelengths identified by H2D-CS were subsequently employed in the development of multiple linear regression (MLR) calibrations for the quantitative analysis of ternary sugar solutions.

MATERIALS AND METHODS

Samples, Chemicals, and Reagents. D-Galactose, D-fructose, and α -D-glucose were obtained from Sigma-Aldrich (Milwaukee, WI). All other reagents used were of analytical grade and obtained from Fisher Scientific (Montreal, QC, Canada). Binary aqueous solutions of glucose/galactose, glucose/fructose, and fructose/galactose having a total sugar concentration of 20% (w/w) were prepared for H2D-CS analysis. Calibration standards consisting of ternary aqueous solutions of the same sugars having a total sugar concentration of 20% (w/w) were prepared according to a 16-point mixture design (Figure 1), which statistically maximizes the information content of the spectra. Twelve additional ternary solutions having a total sugar concentration of 20% (w/w), with the concentrations of fructose, glucose, and galactose being varied randomly over the range of 4–10%, were prepared to serve as a validation set.

Instrumentation. Fourier transform mid-infrared (FTIR) and near-infrared (FTNIR) spectra of sugar solutions were recorded using an ABB Bomem (Québec City, QC, Canada) MB-150 dual-range (NIR/mid-IR) FTIR spectrometer. The spectrometer was purged with dry air from a Balston air drier (Lexington, MA) to minimize spectral contributions from atmospheric water vapor and carbon dioxide. For FTNIR analysis, a 500 μ m quartz transmission cell was used and spectra were recorded from 2850 to 800 nm with a resolution of 16 cm^{-1} and

ratioed against an open-beam background spectrum. For FTIR analysis, a ZnSe single-bounce attenuated total reflectance (SB-ATR) accessory (Harrick, Ossining, NY) was used and spectra were recorded from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} and ratioed against a background single-beam spectrum of the dry clean ZnSe crystal recorded prior to the loading of each sample. The transmission cell and ZnSe crystal were maintained at 30 ± 0.1 °C using Omega (Omega Engineering, Stamford, CT) and Watlow (Watlow System Integrators, Decorah, IA) temperature controllers, respectively, to minimize spectral variability associated with changes in ambient temperature.

H2D-CS Analysis. FTIR and FTNIR spectra of binary sugar solutions were correlated by H2D-CS, a type of generalized 2D correlation spectroscopy described in detail elsewhere (11, 29, 30), using KG2D software (written by Dr. Y. Wang). This software generates 2D contour maps derived from three-dimensional (3D) spectral space. Fourier self-deconvolution (FSD) was employed to enhance the resolution (31) of the FTNIR spectra prior to H2D-CS analysis and was carried out by employing Gplot software (written by D. Moffett) with a k factor of 2 and a bandwidth of 17 cm^{-1} . The contour maps and slice spectra obtained by H2D-CS were examined to select wavelengths for use in MLR calibrations.

Experimental Design, Calibration, and Statistical Analysis. The utility of H2D-CS analysis as a tool for wavelength selection in the development of MLR-based NIR calibrations was evaluated by comparison with a conventional approach whereby wavelengths were selected by examining the first and second derivatives of the spectra of aqueous solutions of the individual sugars. For this purpose, the FTNIR spectra of 20% (w/w) aqueous solutions of fructose, glucose, and galactose were recorded, and the spectrum of water scaled by the water molar fraction was subtracted from them. The first and second derivatives were then computed using the Savitzky–Golay algorithm available in the GRAMS/386 software package (Galactic Industries Co., Salem, NH), and the positions of the first- and second-derivative peaks were selected as wavelengths for use in MLR calibrations.

The FTNIR spectra of the 16 ternary mixtures of sugar solutions recorded in duplicate were used for MLR calibration development. MLR calibration was carried out using the Statgraphics statistical software package (STSC Inc., Rockville, MD), and the wavelengths selected by H2D-CS or derivative spectroscopy were subjected to backward stepwise variable selection (BSVS) (32), a method commonly used for wavelength selection for MLR. The performance of the MLR calibrations developed was evaluated on the basis of their residual standard errors and by computing the standard deviation of the differences for accuracy (SDD_a) between the actual (gravimetric) and fitted values for duplicate FTNIR analyses of the samples in the validation set. To compare the performances of the various calibration approaches, an F test using the variances (s^2) between the actual and predicted values of the calibration and validation sets was employed with a confidence interval of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Spectral Analysis of Sugars. To employ mid-IR spectral information of sugars to gain a better understanding of their NIR spectra using H2D-CS, mid-IR wavelengths characteristic of the constituents of interest need to be selected. In the mid-IR spectrum, the region between 1200 and 950 cm^{-1} provides the most intense and characteristic absorptions of sugars, assigned to coupled C–O and C–C stretching vibrations (33). Figure 2A presents the SB-ATR/FTIR spectra of 20% (w/w) solutions of glucose, fructose, and galactose, illustrating that although their bands over the 1200–950 cm^{-1} region are highly overlapped, each sugar has a distinct spectral profile that differentiates it from the others. Band maxima of fructose, galactose, and glucose at 1062, 1147, and 1032 cm^{-1} , respectively, were used in the H2D-CS analysis to identify corresponding wavelengths in the NIR region for each sugar.

The most intense bands in the NIR spectra of carbohydrates occur in the first combination region (1850–2500 nm), contain-

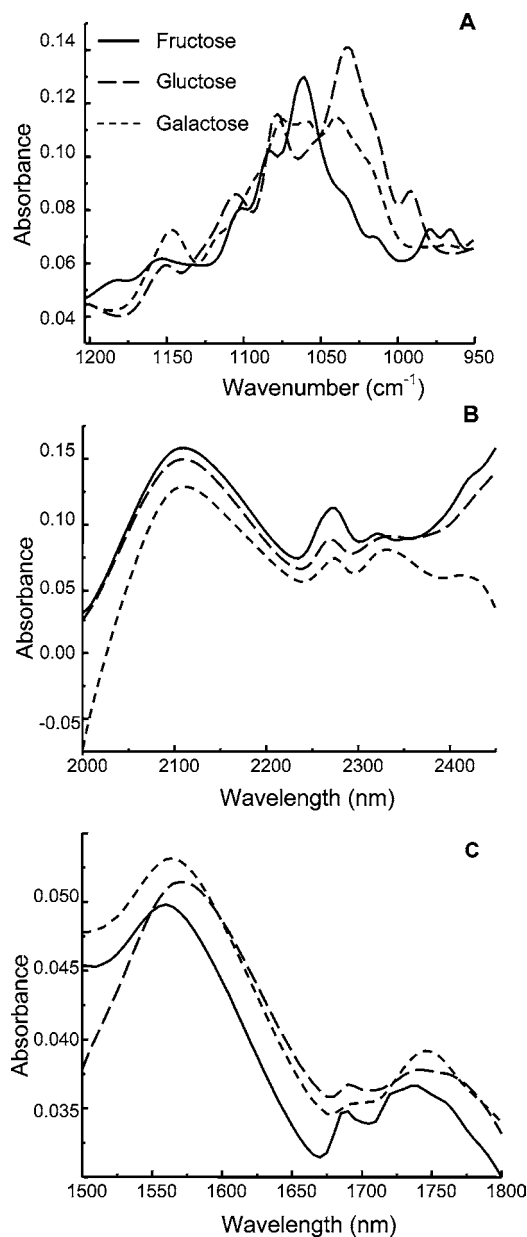


Figure 2. SB-ATR/FTIR spectra for the fingerprint region (from 1200 to 950 cm⁻¹) (A) and FTNIR spectra for the first combination region (B) and the first overtone region (C) of 20% (w/w) solutions of glucose, fructose, and galactose, with the water spectrum subtracted.

ing bands due to C–H stretching plus C–H deformation (2280–2500 nm) and O–H stretching plus O–H deformation (1850–2280 nm), and the first overtone region, containing the first C–H stretching (1650–1850 nm) and first O–H stretching (1350–1650 nm) overtones (34). In aqueous solution, however, two strong water absorption bands at 1450 and 1940 nm effectively blank out sugar spectral features from 1350 to 1480 nm and from 1850 to 2000 nm, respectively. Panels B and C of Figure 2 show the remaining portions of the first combination and first overtone regions, respectively, of the NIR spectra of glucose, galactose, and fructose aqueous solutions [20% (w/w)], with the spectrum of water subtracted. The NIR spectral profiles of glucose, galactose, and fructose are very similar, with bands at 2110, 2273, 2330, 1560, 1740, and 1690 nm. The resolution of the spectra can be enhanced through the use of FSD or by computing their first and second derivatives. As illustrated in Figure 3, when these transforms are carried out on the spectrum

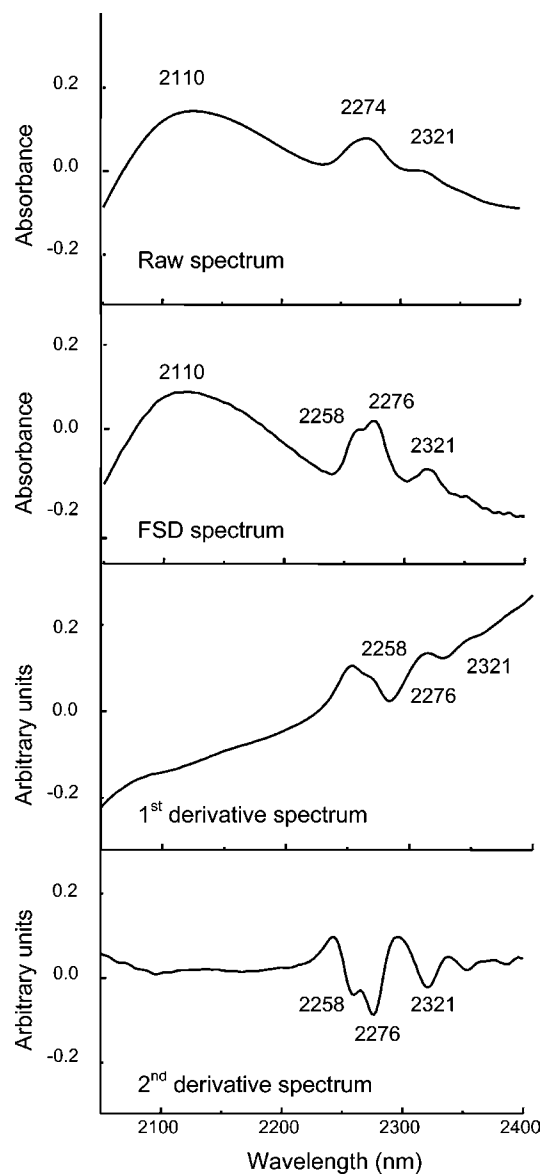


Figure 3. Raw, FSD, and first- and second-derivative FTNIR spectra of a 20% (w/w) fructose solution, with the water spectrum subtracted, in the first combination region.

of a 20% (w/w) fructose solution, they reveal an additional peak at 2258 nm not evident in the raw absorbance spectrum. FSD of the spectra of the 20% (w/w) solutions of glucose and galactose also provided additional characteristic bands for each sugar. In view of the utility of FSD in improving the definition of features on 2D contour maps (28), H2D-CS analysis was carried out on the FSD rather than the raw FTNIR spectra.

H2D-CS Analysis. An H2D-CS examination of the spectra of the binary sugar solutions was carried out over the 1200–950 cm⁻¹ region of the mid-IR spectra relative to the first combination and first overtone regions in the FSD/FTNIR spectra. In each of the three series of binary solutions examined (fructose/galactose, fructose/glucose, and glucose/galactose) the concentration of one sugar increased while that of the other decreased, with the total sugar concentration maintained at 20% (w/w). Panels A–C of Figure 4 show the H2D-CS contour maps generated for fructose/galactose, fructose/glucose, and glucose/galactose solutions, respectively, using the first combination region; the 2400–2500 and 2000–2050 nm ranges have been omitted from the maps as they did not contain any significant spectral information. The corresponding H2D-CS

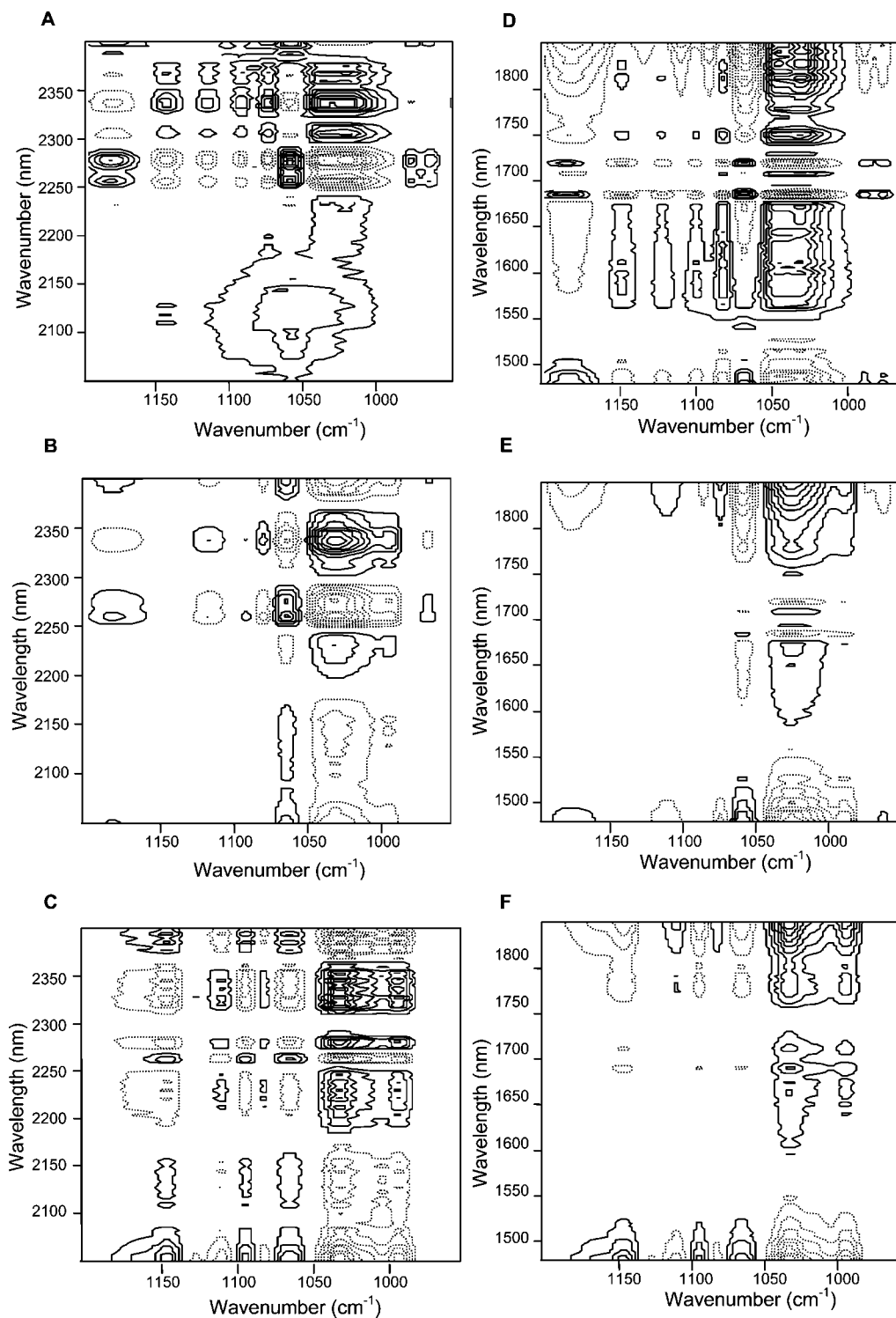


Figure 4. H2D-CS contour maps for (A) fructose/galactose, (B) fructose/glucose, and (C) glucose/galactose solutions obtained by correlation of the first combination region in the FTNIR spectra to the 1200–950 cm^{-1} region in the mid-IR spectra and for (D) fructose/galactose, (E) fructose/glucose, and (F) glucose/galactose solutions obtained by correlation of the first overtone region in the FTNIR spectra to the 1200–950 cm^{-1} region in the mid-IR spectra.

contour maps for the first overtone region are illustrated in panels D–F of **Figure 4**, respectively. The appearance of a contour on a map, termed a crosspeak, indicates a positive correlation between the mid-IR and FSD-NIR spectra if the contour lines are solid, that is, the spectral intensity changes are from the same component, whereas a broken contour line indicates that the spectral intensity changes are from the increase in concentration of one component and the decrease in concentration of the other. **Figure 4A** reveals a positive

correlation between the mid-IR band at 1062 cm^{-1} , corresponding to fructose, and NIR absorption at wavelengths of 2098, 2258, and 2278 nm, as well as a positive correlation between the mid-IR band at 1147 cm^{-1} , corresponding to galactose, and NIR absorption at wavelengths of 2306, 2336, and 2368 nm. Although 2D correlation contour maps representing 3D spectral space can be used to assign peaks, 2D slices of the contour map provide more readily interpretable information about the relationships between a band in one spectrum and multiple bands

Table 1. Signs of H2D-CS Peaks for Fructose/Galactose, Fructose/Glucose, and Galactose/Glucose Binary Solutions^a

λ NIR (nm)	fructose/galactose		fructose/glucose		galactose/glucose	
	fructose (1062 cm ⁻¹)	galactose (1147 cm ⁻¹)	fructose (1062 cm ⁻¹)	glucose (1032 cm ⁻¹)	galactose (1147 cm ⁻¹)	glucose (1032 cm ⁻¹)
1589	-	+	0	0	0	0
1671	-	+	0	0	0	0
1673	0	0	-	+	0	0
1686	+	-	+	-	0	0
1692	0	0	-	+	-	+
1701	0	0	+	-	0	0
1710	0	0	-	+	-	+
1721	+	-	+	-	0	0
1736	0	0	+	-	0	0
1750	-	+	+	+	0	0
1780	0	0	-	+	-	+
1811	-	+	0	0	0	0
1826	-	+	0	0	0	0
2098	+	-	+	-	0	0
2141	0	0	0	0	+	-
2230	0	0	-	+	-	+
2258	+	-	+	-	0	0
2263	0	0	0	0	+	-
2278	+	-	+	-	0	0
2282	0	0	0	0	-	+
2306	-	+	0	0	0	0
2324	0	0	0	0	-	+
2336	-	+	0	0	0	0
2337	0	0	-	+	-	+
2368	-	+	0	0	0	0
2395	0	0	0	0	+	-

^a A positive (+) sign indicates that the mid-IR and FSD-NIR bands are from the same sugar, a negative (-) sign means that the two bands are of different origin, and a "0" means that there is no correlation.

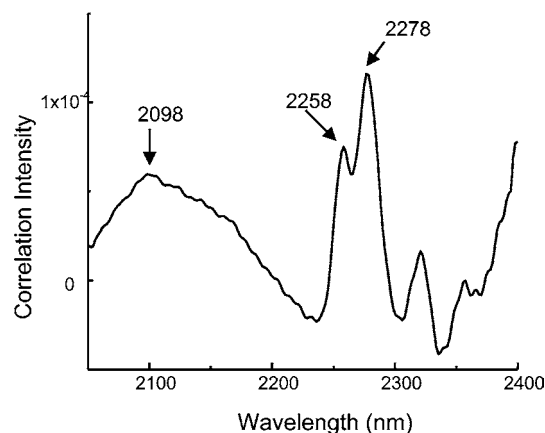


Figure 5. H2D-CS slice spectrum showing correlation between the first combination region in the FTNIR spectra of fructose/galactose solutions and mid-IR absorption at 1062 cm⁻¹ characteristic of fructose.

in another. Thus, slice spectra were generated to show the correlation between absorption at a particular wavenumber in the mid-IR region and NIR absorption at all wavelengths on the contour map. **Figure 5** presents a slice spectrum illustrating the correlation between the band at 1062 cm⁻¹ in the mid-IR spectrum and the FSD/FTNIR spectra, providing a more conventional and interpretable spectral representation of the crosspeaks for fructose at 2098, 2258, and 2278 nm shown in the 2D correlation map in **Figure 4A**.

Table 1 summarizes the crosspeaks for the three sets of binary solutions obtained by examination of slice spectra. A crosspeak associated with a particular component in the H2D-CS maps for the two sets of solutions containing that component may be regarded as a "characteristic" wavelength for that component in relation to the analysis of mixtures of glucose, fructose, and galactose. On the other hand, a crosspeak associated with a particular component in the H2D-CS map for one of the sets of

Table 2. Wavelengths (nm) Selected by Examination of Second-Derivative Spectra and H2D-CS after BSVS for MLR Calibrations for the Prediction of Glucose, Fructose, and Galactose^a

λ	glucose		fructose		galactose	
	2nd deriv	H2D-CS	2nd deriv	H2D-CS	2nd deriv	H2D-CS
λ_1		1589				1589
λ_2				1686	1690	1686
λ_3				1710		
λ_4				1721		
λ_5	1738	1736	1738	1736	1739	1736
λ_6	1779	1780	1779		1779	1780
λ_7		1811				
λ_8						1826
λ_9			2092			
λ_{10}		2141		2141		2141
λ_{11}	2258	2258				
λ_{12}	2267		2267		2267	2263
λ_{13}	2276	2282	2276	2282	2276	2282
λ_{14}		2306		2306		2306
λ_{15}	2323	2324	2323	2324		

^a Wavelengths in bold are those produced by H2D-CS that were not identified by derivative spectroscopy.

solutions containing that component but not the other indicates that the component is the predominant contributor to absorption at that wavelength only in the presence of one of the other two sugars. Thus, although "characteristic" wavelengths were found for fructose and glucose from the H2D correlations, there were no wavelengths at which galactose absorption was predominant in the H2D correlations for both the fructose/galactose and glucose/galactose solutions.

MLR Calibration and Analysis. The NIR wavelengths identified for each sugar from H2D-CS analysis of the binary solutions were used to develop MLR calibrations based on the spectra of 16 ternary sugar solutions, recorded in duplicate. As is commonly done in the development of MLR calibrations,

Table 3. Coefficients of Multiple Determination (R^2), Residual Standard Errors (RSE), and F Ratios of MLR Regressions Based on First- and Second-Derivative Selected Wavelengths As Compared to MLR Regressions Based on H2D-CS Selected Wavelengths

method	glucose			fructose			galactose		
	R^2	RSE (% w/w)	F ratio ^a	R^2	RSE (% w/w)	F ratio ^a	R^2	RSE (% w/w)	F ratio ^a
MLR (H2D-CS)	0.9983	0.299		0.9991	0.216		0.9969	0.403	
MLR (1st derivative)	0.9964	0.433	2.10	0.9989	0.237	1.21	0.9947	0.527	1.700
MLR (2nd derivative)	0.9969	0.400	1.79	0.9987	0.260	1.45	0.9938	0.570	1.998

^a F ratio [$n = 32$, degrees of freedom (df) = 2, and confidence level of $\alpha = 0.05$] based on the ratio of the variances of the differences between the actual and fitted values. The critical F value is 1.81.

Table 4. SDD_a between Actual and Predicted Values of the Validation Set and F Ratios of MLR Regressions Based on First- and Second-Derivative Selected Wavelengths As Compared to MLR Regressions Based on H2D-CS Selected Wavelengths

method	glucose		fructose		galactose	
	SDD _a (% w/w)	F ratio ^a	SDD _a (% w/w)	F ratio ^a	SDD _a (% w/w)	F ratio ^a
MLR (H2D-CS)	0.423		0.180		0.539	
MLR (1st derivative)	0.612	2.09	0.276	2.34	0.759	1.98
MLR (2nd derivative)	0.526	1.55	0.291	2.61	0.846	2.46

^a F ratio [$n = 24$, degrees of freedom (df) = 2, and confidence level of $\alpha = 0.05$] based on the ratio of the variances of the differences between the actual and predicted values. The critical F value is 2.05.

stepwise variable selection was applied to select optimal combinations of wavelengths; although the use of genetic algorithms for this purpose has recently been demonstrated (35), this alternative approach was not investigated in the present work. **Table 2** lists the wavelengths obtained for the determination of each sugar by application of BSVS. The performance of the resulting MLR calibrations was assessed on the basis of the residual standard errors (**Table 3**) and the agreement between the fitted and actual (gravimetric) values of 12 validation samples analyzed in duplicate, expressed in terms of SDD_a (**Table 4**). For the MLR calibration based on H2D-CS selected wavelengths, an SDD_a of 0.423, 0.180, or 0.539% (w/w) was obtained for glucose, fructose, or galactose, respectively; the poorer results for galactose are consistent with there being less spectral information specific to this component as indicated by the H2D-CS study.

For comparative purposes, MLR calibrations were also developed by applying BSVS to wavelengths selected by examination of the first- and second-derivative spectra of solutions of the individual sugars, and the sets of wavelengths obtained in this manner are also listed in **Table 2**. Overall, these wavelengths are also in the H2D-CS-derived wavelength sets, whereas the wavelengths highlighted in bold in **Table 2** are additional wavelengths present only in the H2D-CS-derived wavelength sets. Thus, the H2D-CS wavelength selection approach allowed additional relevant spectral information to be utilized in the development of the MLR calibrations. A key advantage of the H2D-CS wavelength selection approach is that it provides the capability of taking into account interactions between the components in the sample matrix that result in shifting of band positions as compared to the spectra of the respective pure components (12). However, in a detailed examination of the NIR spectra of pure glucose, fructose, and sucrose solutions as a function of concentration over the range of 5–65% (w/v) reported by Giangiacomo (36), which included the use of 2D correlation analysis to investigate the concentration-dependent shifts in the water and sugar OH absorption bands, the predominance of sugar–water interactions over sugar–sugar interactions at sugar concentrations below 25–30% was noted. Hence, at the concentration [20% (w/w)] employed in the present work, no significant band shifts due to sugar–sugar interactions would be anticipated, and hence it is

not unexpected that the F test results in **Tables 3** and **4** indicate only minor improvements at the 95% confidence interval between MLR calibrations based on wavelength selection using the derivative spectra of pure solutions of individual sugars and the MLR calibrations based on wavelength selection by H2D-CS.

For the purposes of this study, binary sugar solutions in a concentration range in which the principal intermolecular interactions are sugar–water interactions were selected as the simplest model system that could be employed for H2D-CS analysis. Correlation of the FSD/FTNIR and FTIR spectra of these aqueous glucose/galactose, glucose/fructose, and fructose/galactose solutions by H2D-CS provided information on the NIR spectral characteristics of each sugar, and this information was employed in wavelength selection using BSVS for the development of MLR calibrations for the quantitative analysis of ternary aqueous solutions of glucose, fructose, and galactose. In future work, this approach will be extended to H2D-CS analysis of ternary and higher mixtures in which intermolecular interactions have a significant role. For such systems, H2D-CS could be a powerful tool for wavelength selection in NIR calibration development, significantly improving the performance of the calibration models.

ABBREVIATIONS USED

BSVS, backward stepwise variable selection; FSD, Fourier self-deconvolution; FTIR, Fourier transform infrared; FTNIR, Fourier transform near-infrared; H2D-CS, heterospectral two-dimensional correlation spectroscopy; IR, infrared; MLR, multiple linear regression; NIR, near-infrared; SB-ATR, single-bounce attenuated total reflectance; SDD_a, standard deviation of the differences for accuracy.

LITERATURE CITED

- Bertrand, D.; Robert, P. Near infrared spectroscopy. In *Analytical Techniques for Foods and Agricultural Products*; Linden, G., Ed.; VCH Publishers: New York, 1996; pp 59–66.
- Li-Chan, E. C. Y.; Ismail, A. A.; Sedman, J.; van de Voort, F. R. Vibrational spectroscopy of food and food products. In *Handbook of Vibrational Spectroscopy*; Chalmers, J. M., Griffiths, P. R., Eds.; Wiley: Chichester, U.K., 2002; pp 3629–3662.

- (3) Hruschka, W. R. Data analysis: wavelength selection methods. In *Near-Infrared Technology in the Agricultural and Food Industries*; Williams, P., Norris, K., Eds.; American Association of Cereal Chemists: St. Paul, MN, 2001; pp 39–58.
- (4) Giangiacomo, R.; Magee, J. B.; Birth, G. S.; Dull, G. G. Predicting concentrations of individual sugars in dry mixtures by near-infrared reflectance spectroscopy. *J. Food Sci.* **1981**, *46*, 531–534.
- (5) Dumoulin, E. D.; Bernard, A. P.; Guerin, J. T. Determination of sugar and ethanol content in aqueous products of molasses distilleries by near infrared spectrophotometry. *J. Food Sci.* **1987**, *52*, 626–630.
- (6) Rambla, F. J.; Garrigues, S.; de la Guardia, M. PLS-NIR determination of total sugar, glucose, fructose and sucrose in aqueous solutions of fruit juices. *Anal. Chim. Acta* **1997**, *344*, 41–53.
- (7) Giangiacomo, R.; Dull, G. G. Near infrared spectrophotometric determination of individual sugars in aqueous mixtures. *J. Food Sci.* **1986**, *51*, 679–683.
- (8) Sinnaeve, G.; Dardenne, P.; Agneessens, R. Quantitative analysis of raw apple juices using near infrared, Fourier-transform near infrared and Fourier-transform infrared instruments: a comparison of their analytical performances. *J. Near Infrared Spectrosc.* **1997**, *5*, 1–17.
- (9) Garcia-Jares, C. M.; Medina, B. Application of multivariate calibration to the simultaneous routine determination of ethanol, glycerol, fructose, glucose and total residual sugars in botrytized-grape sweet wines by means of near-infrared reflectance spectroscopy. *Fresenius' J. Anal. Chem.* **1997**, *357*, 86–91.
- (10) Robert, P.; Devaux, M. F.; Qannari, A.; Safar, M. Mid and near infrared study of carbohydrates by canonical correlation analysis. *J. Near Infrared Spectrosc.* **1993**, *1*, 99–108.
- (11) Noda, I. Generalized two-dimensional correlation method applicable to infrared, Raman, and other types of spectroscopy. *Appl. Spectrosc.* **1993**, *47*, 1329–1336.
- (12) Ozaki, Y.; Noda, I. Potential of generalized two-dimensional correlation spectroscopy in the near infrared region. *J. Near Infrared Spectrosc.* **1996**, *4*, 85–99.
- (13) Ozaki, Y.; Sasic, S.; Tanaka, T.; Noda, I. Two-dimensional correlation spectroscopy: principle and recent theoretical development. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 1–17.
- (14) Ren, Y.; Matsushita, A.; Matsukawa, K.; Inoue, H.; Minami, Y.; Noda, I.; Ozaki, Y. Two-dimensional Raman and Raman-near infrared heterospectral correlation spectroscopy studies of the specific interaction in partially miscible blends of poly(methyl methacrylate) and poly(4-vinyl phenol). *AIP Conf. Proc.* **2000**, *503*, 250–253.
- (15) Matsushita, A.; Ren, Y.; Matsukawa, K.; Inoue, H.; Minami, Y.; Noda, I.; Ozaki, Y. Two-dimensional Fourier-transform Raman and near-infrared correlation spectroscopy studies of poly(methyl methacrylate) blends 1. Immiscible blends of poly(methyl methacrylate) and atactic polystyrene. *Appl. Spectrosc.* **2000**, *54*, 171–180.
- (16) Czarnecki, M. A.; Wu, P.; Siesler, H. W. 2D FT-NIR and FT-IR correlation analysis of temperature-induced changes of nylon 12. *Chem. Phys. Lett.* **1998**, *283*, 326–332.
- (17) Amari, T.; Ozaki, Y. Generalized two-dimensional attenuated total reflection/infrared and near-infrared correlation spectroscopy studies of real-time monitoring of the oligomerization of bis(hydroxyethyl terephthalate). *Macromolecules* **2002**, *35*, 8020–8028.
- (18) Noda, I.; Liu, Y.; Ozaki, Y. Two-dimensional correlation spectroscopy study of temperature-dependent spectral variations of *N*-methylacetamide in the pure liquid state. 2. Two-dimensional Raman and infrared-Raman heterospectral analysis. *J. Phys. Chem.* **1996**, *100*, 8674–8680.
- (19) Jung, Y. M.; Czarnik-Matusiewicz, B.; Ozaki, Y. An IR vs. Raman 2D heterospectral correlation study of the secondary structure of β -lactoglobulin in aqueous solutions. *AIP Conf. Proc.* **2000**, *503*, 275–278.
- (20) Kubelka, J.; Pancoska, P.; Keiderling, T. A. Novel use of a static modification of two-dimensional correlation analysis. Part II: Hetero-spectral correlations of protein Raman, FT-IR, and circular dichroism spectra. *Appl. Spectrosc.* **1999**, *53*, 666–671.
- (21) Barton, F. E., II; Himmelsbach, D. S.; Duckworth, J. H.; Smith, M. J. Two-dimensional vibration spectroscopy: correlation of mid- and near-infrared regions. *Appl. Spectrosc.* **1992**, *46*, 420–429.
- (22) Barton, F. E., II; Himmelsbach, D. S. Two-dimensional vibrational spectroscopy II: correlation of the absorptions of lignins in the mid- and near-infrared. *Appl. Spectrosc.* **1993**, *47*, 1920–1925.
- (23) Barton, F. E., II; Himmelsbach, D. S.; McClung, A. M.; Champagne, E. L. Two-dimensional vibration spectroscopy of rice quality and cooking. *Cereal Chem.* **2002**, *79*, 143–147.
- (24) Barton, F. E., II; Himmelsbach, D. S.; Archibald, D. D. Two-dimensional vibrational spectroscopy V: correlation of mid- and near-infrared of hard red winter and spring wheats. *J. Near Infrared Spectrosc.* **1996**, *4*, 139–152.
- (25) McClure, W. F.; Maeda, H.; Dong, J.; Liu, Y.; Ozaki, Y. Two-dimensional correlation of Fourier transform near-infrared and Fourier transform Raman spectra I: mixtures of sugar and protein. *Appl. Spectrosc.* **1996**, *50*, 467–475.
- (26) Awichi, A.; Tee, E. M.; Srikanthan, G.; Zhao, W. Identification of overlapped near-infrared bands of glucose anomers using two-dimensional near-infrared and middle-infrared correlation spectroscopy. *Appl. Spectrosc.* **2002**, *56*, 897–901.
- (27) Maalouly, J.; Eveleigh, L.; Rutledge, D. N.; Ducauze, C. J. Application of 2D correlation spectroscopy and outer product analysis to infrared spectra of sugar beets. *Vibr. Spectrosc.* **2004**, *36*, 279–285.
- (28) Ismoyo, F.; Wang, Y.; Ismail, A. A. Examination of the effect of heating on the secondary structure of avidin and avidin-biotin complex by resolution-enhanced 2D infrared correlation spectroscopy. *Appl. Spectrosc.* **2000**, *54*, 939–947.
- (29) Noda, I. Two-dimensional infrared (2D IR) spectroscopy: theory and applications. *Appl. Spectrosc.* **1990**, *44*, 550–561.
- (30) Noda, I.; Dowrey, A. E.; Marcott, C. Recent developments in two-dimensional infrared (2D IR) correlation spectroscopy. *Appl. Spectrosc.* **1993**, *47*, 1317–1323.
- (31) Kauppinen, J. K.; Moffatt, D. J.; Cameron, D. G.; Mantsch, H. H. Fourier self-deconvolution: a method for resolving intrinsically overlapped bands. *Appl. Spectrosc.* **1981**, *35*, 271–276.
- (32) Osborne, B. G.; Fearn, T. Near infrared data handling and calibration by multiple linear regression. In *Near Infrared Spectroscopy in Food Analysis*; Longman Scientific and Technical: Essex, U.K., 1986; pp 86–103.
- (33) Mathlouthi, M.; Koenig, J. L. Vibrational spectra of carbohydrates. *Adv. Carbohydr. Chem. Biochem.* **1986**, *44*, 7–89.
- (34) Robert, C.; Cadet, F. Analysis of near-infrared spectra of some carbohydrates. *Appl. Spectrosc. Rev.* **1998**, *33*, 253–266.
- (35) Broadhurst, D.; Goodacre, R.; Jones, A.; Rowland, J. J.; Kell, J. B. Genetic algorithms as a method for variable selection in multiple linear regression and partial least squares regression, with applications to pyrolysis mass spectrometry. *Anal. Chim. Acta* **1997**, *348*, 71–86.
- (36) Giangiacomo, R. Study of water-sugar interactions at increasing sugar concentration by NIR spectroscopy. *Food Chem.* **2006**, *96*, 371–379.

Received for review January 27, 2006. Revised manuscript received July 7, 2006. Accepted July 17, 2006.

JF060266A