A Rapid Spectroscopic Technique for Determining Honey Adulteration with Corn Syrup

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ABSTRACT: A combination of Fourier transform infrared spectroscopy with multivariate procedures was used for determining the level of sugar addition to honey. Spectra of honey adulterated with different levels of glucose, fructose, sucrose, and corn syrup were recorded in the mid–infrared range using the attenuated total reflectance accessory. The standard error of prediction (SEP) in validation set was between 1.99% and 2.22% using partial least square (PLS) on first derivative transformed data. Results showed that the combined model for 3 different varieties of honey gave lower correlation. It is demonstrated that Fourier transform infrared spectroscopy has good potential for detecting corn-syrup adulteration in honey in less than 5 min.

Keywords: adulteration, honey, Fourier transform infrared spectroscopy, attenuated total reflectance accessory, multivariate statistics, partial least square regression, principal component regression

Introduction

HONEY HAS A WIDE RANGE OF APPLICATIONS IN THE FOOD industry. It can be processed for direct consumption or used as an ingredient in various processed food products. Because of its nutritional value and unique flavor, the price of natural bee honey is much higher than that of other sweeteners, such as refined cane sugar, beet sugar, and corn syrup. The authenticity of honey is of great importance for commercial and health reasons. Adulteration of bee honey with cheaper sweetening materials has been reported in the literature (Cienfuegos and others 1997; Tien and Shau 1997; Gonzalez and others 1998).

Authentication is a wide-ranging issue and has come to prominence in recent years. Regulatory authorities, food processors, retailers, and consumer groups all have an interest in ensuring that foods are what they are purported to be. Many foods have the potential to be naturally or deliberately adulterated, but those that are expensive and are produced under wide fluctuations in weather and harvesting conditions are particularly susceptible. Honey is one such material, especially so in recent times because of the increasing practice of selling honey on the basis of varietal and/or geographic origin. Surveys of honey composition have established that the 3 major components are fructose, glucose, and water (White and others 1962). Table 1 shows the average values of the constituents of honey from 490 samples. Stable carbon isotope ratio analysis of honey to detect the undeclared presence of cane or corn sugars is a standard technique researched during the past 20 y (White and others 1998). This method is time-consuming and expensive. The cost of analyzing samples for adulteration range currently is U.S. $50 for a test to identify the presence of cane or corn sugar using the carbon isotope method (GAO Report 1995). However, this procedure can only detect levels between 10% and 20%. A solution to this problem is to use a very rapid and inexpensive analytical screening tool that could detect a much wider range of different compounds in one sample run. Suspected samples could be selectively screened and evaluated by more rigorous methods such as carbon isotope ratio analysis. While a number of approaches based on HPLC, NMR spectroscopy, and carbon isotope methodology have been reported (Vogels and others 1996; Ciolino 1998; Martin and others 1998), those based on spectroscopic techniques have much to offer because of their ease of use, rapidity, and low cost (Kemsley and others 1996; Downey and others 1997; Jowder and others 1999).

Table 1—Average composition of 490 samples of honey and range of values (White and others 1962)

Component	Average	Standard deviation	Range
Moisture, %	17.2	1.46	13.4-22.9
Fructose, %	38.19	2.07	27.25-44.26
Glucose, %	31.28	3.03	22.03-40.75
Sucrose, %	1.31	0.95	0.25-7.57
Maltose, %	7.31	2.09	2.74-15.98
Higher sugars, %	1.50	1.03	0.13-8.49
Free acid, meq/kg	22.03	8.22	6.75-47.19
Lactone, meq/kg	0.335	0.135	0.0-0.95
Ash, %	0.169	0.15	0.02-1.028
Nitrogen, %	0.041	0.026	0.0-0.133
Diastase value	20.8	9.76	2.1-61.2

Infrared spectroscopy represents an attractive option for quality screening because it is rapid, inexpensive, and noninvasive. Mid-infrared (MIR) spectroscopy, in particular, rapidly provides information on a very large number of analytes, and the absorption bands are sensitive to the physical and chemical states of individual constituents. The high spectral signal-to-noise ratio obtained from modern instrumental analysis allows the detection of constituents present in very low concentrations, as well as subtle compositional differences between and among multiconstituent specimens. Fourier transform infrared (FTIR) spectroscopy is one such technique (Wilson and Goodfellow 1994). It can be thought of as a molecular "fingerprinting" method. An infrared spectrum contains features arising from vibrations of molecular bonds, and the mid-infrared region (400 to 4000 cm^{-1}) in particular is highly sensitive to the precise chemical composition of a sample. FTIR has been shown to be useful for a range of identification problems in the food sector (Lai and others 1994; Briandet and others 1996), including the adulteration of fruit purees (Kemsley and others 1996). Depend-

ing upon the concentration, this technique can quantitatively and simultaneously detect all liquid sugar compounds in a sample with the help of attenuated total reflectance (ATR) accessory. The combination of mid-infrared spectroscopy and multivariate statistics for determining glucose, fructose, and sucrose in aqueous mixtures was investigated (Sivakesava and Irudayaraj 2000). Calibration methods developed with PLS and PCR gave an average standard error of calibration values of 0.18% and 0.21% (w/w), respectively. The values of correlation coefficient were generally satisfactory ($R^2 = 0.9$) for all calibrations.

The application of FTIR-ATR spectroscopy will, however, generate spectra that are either complex or visually similar to accentuate the subtle differences in the qualitative characteristics. A solution to this problem is to analyze the data by multivariate methods such as partial least squares (PLS) (Haaland and Thomas 1988) and principal component regression (PCR) (Martens and Naes 1989) to develop prediction models. Such methods also allow quantitative multicomponent analysis in mixtures. A comparative study by Dupuy and others (1992) of different multivariate calibration methods applied to the determination of glucose, fructose, and sucrose in dried fruit juice extracts showed that PLS gives the most accurate results.

In this paper, the application of FTIR-ATR spectroscopy for the detection of adulteration by corn syrup in 3 varieties of honey is described. Among the many different adulterant materials, corn syrup is a potential one. ATR spectra were calibrated with different percentages of corn syrup added to honey. PLS and PCR mathematical treatments for the description and processing of MIR spectral data of adulterated honey samples were evaluated. Development of a rapid detection technique will be of practical significance to the processes in quality assurance programs. The specific objective of this research is to develop a simple and rapid spectroscopic procedure together with suitable chemometric models to detect corn-syrup adulteration in selected honey varieties.

Materials and Methods

Samples

Two types of calibration sets were prepared. The first was honey (orange blossom; University Creamery, The Pennsylvania State Univ., University Park, Pa., U.S.A.) adulterated with sugar mixtures comprised of glucose, fructose, and sucrose (purity > 99%; Staley, Decatur, Ill., U.S.A.). In the 2nd set, 3 different varieties of honey were adulterated with corn syrup.

The multicomponent adulteration set included 65 solution mixtures covering a total sugar concentration range from 5% to 25% (w/w). The ranges were chosen to evaluate the adequacy of the method for the adulteration of honey. Forty-five samples were prepared with each of the sugars (glucose, fructose, and sucrose) ranging from low to high values of concentration for each and in different ratios with the other 2 (Table 2). Twenty samples were separately prepared for validation using a similar technique as in Table 2. Prior to spectroscopic analysis, honey samples were incubated in a water bath at about 50 °C until all sugar crystals were no longer visible. The samples were then mixed and kept at room temperature to equilibrate before FTIR analysis.

In the 2nd phase of the study, orange blossom (University Creamery, The Pennsylvania State Univ.), clover, and buckwheat (Rebuck Apiaries, Montoursville, Pa., U.S.A.) varieties of honey were mixed with different quantities of corn syrup.

Table 2—Concentrations of glucose, fructose, and sucrose in calibration mixtures

Sample	Glucose % (w/w)	Fructose % (w/w)	Sucrose % (w/w)	Total sugar concentration % (w/w)
1	0.5	4.0	0.5	5
2	1	3.0	1.0	5
3	1.5	2.0	1.5	5
4	2.0	1.5	1.5	5
5	2.5	1.5	1.0	5
6	3.0	1.0	1.0	5
7	3.5	1.0	0.5	5
8	1.0	2.0	5.0	8
9	2.0	3.0	3.0	8
10	3.0	1.0	4.0	8
11	2.0	5.0	1.0	8
12	6.0	1.5	2.5	10
13	5.0	2.0	3.0	10
14	1.5	1.5	7.0	10
15	2.0	3.0	5.0	10
16	2.5	1.5	6.0	10
17	6.0	2.5	1.5	10
18	1.5	7.0	1.5	10
19	3.0	5.0	2.0	10
20	2.5	6.0	1.5	10
21	1.5	2.5	6.0	10
22	3.0	5.0	4.0	12
23	5.0	2.0	5.0	12
24	7.0	3.0	2.0	12
25	9.0	1.0	2.0	12
26	2.0	7.0	3.0	12
27	1.0	4.0	7.0	12
28	5.0	5.0	5.0	15
29	3.0	8.0	4.0	15
30	7.0	7.0	1.0	15
31	10.0	1.0	4.0	15
32	12.0	2.0	1.0	15
33	1.0	10.0	4.0	15
34	1.0	12.0	2.0	15
35	12.0	3.0	5.0	20
36	10.0	4.0	6.0	20
37	3.0	3.0	14.0	20
38	4.0	6.0	10.0	20
39	5.0	3.0	12.0	20
40	12.0	5.0	3.0	20
41	3.0	14.0	3.0	20
42	10.0	10.0	5.0	25
43	5.0	12.0	8.0	25
44	3.0	8.0	14.0	25
45	8.0	14.0	3.0	25

The adulteration set included 51 samples for each of the 3 honey varieties adulterated with corn syrup (CPC International Inc., Englewood Cliffs, N.J., U.S.A.) in the range between 2% and 27% (w/w) in steps of 0.5%. These are representative of levels at which adulteration may occur. Up to 39 of these were used for calibration, and the remaining 12 were used for validation.

FTIR

All spectra were collected on a Bio-Rad (Cambridge, Mass., U.S.A.) FTS 6000 spectrometer operating in the mid-infrared (400 to 4000 cm^{-1}) region. The instrument was fitted with a sealed and desiccated interferometer with a deuterated triglycine sulfate (DTGS) detector. The sampling station was equipped with an overhead attenuated total reflection (ATR) accessory, comprised of transfer optics within the chamber through which the infrared radiation was directed

to a detachable ATR crystal. The zinc selenide crystal was mounted into a plate with a shallow trough for sample containment. The crystal geometry was a 45° parallelogram with mirrored angled faces with nominal 10 internal reflections. The depth of penetration that gives a measure of the intensity of the resulting spectrum is 1.46 microns. All spectral measurements were made at 32 cm^{-1} resolution, with 256 interferograms co-added before Fourier transformation. The background spectrum was recorded using distilled water. Single-beam ATR spectra were collected from each sample and transformed to absorbance units using a background spectrum of distilled water. The ATR crystal was carefully cleaned between analysis with water and dried with nitrogen gas. The cleaned crystal was checked spectrally to ensure that no residue from the previous sample was retained on the crystal surface. Spectra were collected in duplicate and used in calibration and validation studies.

Chemometric Operations

The GRAMS 32 software (Galactic Industries Corp., Salem, N.H., U.S.A.) used for quantitative analysis employed the partial least squares (Haaland and Thomas 1988) and principal component regression algorithms (Martens and Naes 1989). Calibration models were developed with spectra in absorbance units by the use of PLS and PCR analysis with original and first derivative spectra. An optimum number of factors for calibrations were selected based on the predicted residual sum of squares (PRESS), along with the R^2 from regression. The software was used to find the correlation coefficient between predicted and actual values. The prediction ability of the models was tested by computing the standard error of prediction (SEP):

$$SEP = \sqrt{\frac{\sum_{i=1}^{n} (actual\ concentration - predicted\ concentration)^2}{n}}$$

The term "actual concentration" refers to the sugar concentration added to the specific honey sample, "predicted concentration" refers to a concentration value computed using spectral data, and "n" is the number of samples in the calibration set. Cross-validation was used in all cases to minimize the risk of over-fitting the calibrations when evaluating calibration accuracy.

Mathematical enhancements are normally applied to data to remove redundant information and enhance the important sample-to-sample differences that exist within the data. Two different data enhancement methods were used. The first is termed "mean centering" and involves calculating the average spectrum of all the spectra in the training set and then subtracting the result from each spectrum. In addition, the mean concentration value for each constituent is calculated and subtracted from the concentrations of every sample. By removing the mean from the data, differences between the samples are substantially enhanced in terms of both concentration and spectral response. This usually leads to calibration models that provide more accurate predictions.

The 2nd method is called "variance scaling" and emphasizes small variations in the data by giving equal weighting to all values. Variance scaling is calculated by dividing the response at each spectral data point by the standard deviation of the responses of all training spectra at that point. By giving equal weighting to all the information in the data, calibration errors in the model are more consistent across all constituents. The performances of the established calibration methods were further validated using the samples in the validation set. The predicted values were correlated with the actual values, and the accuracy of prediction was assessed by calculating the standard error of prediction (SEP).

Results and Discussion

Characterization of Honey Spectrum

Single-beam spectra were obtained for all samples and corrected against the background spectrum of water to present the spectra in absorbance units. In the analysis of aqueous solutions, overlap of the vibrational bands of water with those of the solutes is inevitable, resulting in broad bands that cannot usually be deconvoluted into their constituents. In this case, MIR spectroscopy can be made more sensitive and accurate by using the spectrum of water as background.

Figure 1 presents the ATR spectra of pure honey with the corresponding band assignments. Spectra of honey show absorbance bands at 774, 913, 1017, 1249, 1349, 1413, 2908, and 3284 cm^{-1}. These bands are representative of the chemical groups of components present in the sample. Assignment of functional groups corresponding to the vibration modes was based on identification of the spectrum peaks and matching the frequency with the corresponding chemical group that absorbs in the MIR region. The region 800 to 1500 cm^{-1} corresponds to the absorption zones of the 3 major sugar constituents of honey: fructose, glucose, and sucrose. The 900 to 750 cm^{-1} region is the anomeric region and is characteristic of the saccharide configurations (Tul'chinsky and others 1976). The bands in the 904 to 1153 cm^{-1} region are assigned to C-O and C-C stretching modes (Hineno 1977), and those around 1474 to 1199 cm^{-1} are due to the bending modes of O-C-H, C-C-H, and C-O-H angles. Negative bands were observed around 1618 and 3635 cm^{-1}. These bands are due to lower water concentration in the honey sample compared with the reference employed and the fact that water presents an O-H stretching overtone at these wavelengths (Chen and Irudayaraj 1998). A broad, strong NH_3^+ stretching band in the 3000 to 2700 cm^{-1} region can be related to primary amino acids (Silverstein and Webster 1998).

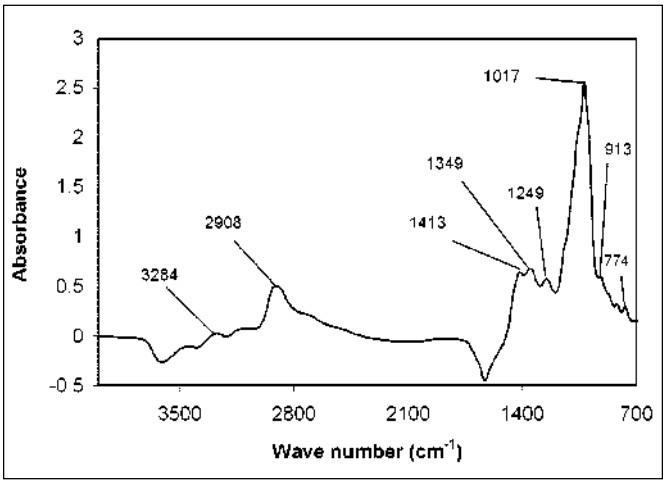

Figure 1—ATR spectrum of pure honey

Table 3—Calibration parameters for sugar mixtures in honey

Calibration method	Factors[a]			R^{2b}			SEP[c]		
	Glucose	Fructose	Sucrose	Glucose	Fructose	Sucrose	Glucose	Fructose	Sucrose
Mean-centering									
PLS	15	12	11	0.901	0.921	0.918	1.041	0.861	1.034
PLS-first derivative	16	15	10	0.904	0.948	0.919	0.984	0.784	1.015
PCR	15	15	15	0.853	0.934	0.909	1.277	0.887	1.088
PCR-first derivative	20	20	20	0.858	0.945	0.914	1.205	0.831	1.052
Variance Scale									
PLS	15	14	10	0.903	0.946	0.924	1.022	0.809	0.977
PLS-first derivative	8	9	6	0.913	0.937	0.916	0.931	0.872	1.041
PCR	14	14	14	0.892	0.944	0.925	1.078	0.767	1.001
PCR-first derivative	17	17	17	0.926	0.935	0.922	0.948	0.899	1.082

a Optimum number of factors
b Correlation coefficient
c Standard error of prediction (%, w/w)

Table 4—Validation parameters for sugar mixtures in honey using different calibration methods

Calibration method	Correlation coefficient, R^2			SEP, % (w/w)		
	Glucose	Fructose	Sucrose	Glucose	Fructose	Sucrose
Mean centering						
PLS	0.786	0.933	0.896	2.700	1.015	1.654
PLS-first derivative	0.812	0.939	0.917	2.650	1.003	1.546
PCR	0.728	0.904	0.891	3.112	1.289	1.910
PCR-first derivative	0.807	0.938	0.910	3.040	1.013	1.780
Variance scale						
PLS	0.834	0.937	0.904	2.144	1.144	1.618
PLS-first derivative	0.862	0.942	0.919	1.687	0.942	1.443
PCR	0.791	0.924	0.903	1.989	1.296	1.787
PCR-first derivative	0.829	0.940	0.912	1.720	1.237	1.663

Selection of Spectral Region for Calibration

An important parameter used in computing a multivariate calibration method is the spectral range supplied during model building. If there are regions in the spectrum with very strong absorption peaks (thus nonlinear with respect to Beer's Law), it is usually best to choose the regions on either side, thus excluding that band. While PLS and PCR factor analysis can correct for some nonlinearities, they cannot correct for regions of over-absorbance. Suitable spectral ranges can be identified by computing the correlation spectrum between mixture of sugars and corn-syrup concentrations and absorbance (Figure 2a and 2b). Regions that show high correlation should be selected for multivariate analysis. Figure 2a and 2b show high correlation bands between 800 and 1500 cm^{-1} and are due to the sugars (Cadet and Offmann 1997) and hence selected for calibration.

Calibration of Honey Adulteration with Sugar Mixtures

Preliminary experiments were conducted with the orange blossom variety of honey adulterated with pure solutions of glucose, fructose, and sucrose. The calibration methods developed gave average R^2 values of 0.996, 0.95, and 0.884 for glucose, fructose, and sucrose, respectively. Results showed that the spectroscopic technique could predict the contents of glucose, fructose, and sucrose in honey.

MIR spectra of honey adulterated with sugar mixtures were collected. The spectral data for each calibration set were subjected to multivariate analysis for the development of calibration methods for each sugar. The value of R^2 and the factors included in the calibration methods and the SEP values for glucose, fructose, and sucrose using different calibration methods are presented in Table 3. In general, SEP values of fructose are less compared to glucose and sucrose. The PLS first derivative calibration method with variance scale data processing used less factors (8, 9, and 6 for glucose, fructose, and sucrose, respectively). The calibration tests with PLS and PCR showed that PLS performed better by considering all the results. Calibration with these methods captures as much of the variation in the whole spectral range as possible (Osborne and others 1993). However, PCR does not consider the reference values when selecting or constructing spectral components, whereas in the PLS regression model, information about reference values is used in constructing spectral components (Osborne and others 1993). This may explain why PLS performed better than the PCR model.

Validation tests showed that the R^2 for variance scale data processing is relatively high for PLS and PCR methods (Table 4). The data also show that the error in predicting the sugar concentration decreased with first derivative data transformation using variance scale data processing. Although glucose, fructose, and sucrose have similar structures and all contain C-H and O-H bonds, subtle differences exist in their MIR absorption spectra. The R^2 values between the content of individual sugars and the contents predicted by the MIR calibration equation were high, further indicating the specificity of the MIR calibration equations for individual sugars.

Calibration of Adulteration with Corn Syrup

There was no certainty that changes in honey sugar composition in products from different honey varieties would be

sufficiently narrow to allow discrimination. In general, honey sugar composition varies from one variety to another. Three different varieties of honey (buckwheat, clover, and orange blossom) were used in this study. Calibration was carried out with samples of genuine honey spiked with corn syrup in the range between 2% and 27% (w/w) in increments of 0.5%, which are representative of levels at which adulteration may occur in a commercial setting. The addition of sugars to genuine honey constitutes adulteration and is expressed as adulteration percent.

The factors included in the calibration methods and the SEP values for different calibration methods using the mean centering data processing method are given in Table 5. In general, R^2 values are high (> 0.9) for all the methods used. The first derivative mathematical pretreatment of spectra gave slightly smaller values of SEP and used fewer factors for the calibration (Table 5). Derivative transformations could partially compensate for baseline offset between samples and reduce instrument drift effects (Norris 1982). The results using variance scale data enhancement are not shown since the prediction was not improved.

The calibrations were then applied to the corresponding validation data sets for the computation of added corn-syrup concentrations in honey samples. Validation results showed that the spectroscopic technique could predict the contents of corn syrup in all 3 varieties of honey (Table 5). Correlation between the predicted values and the actual values had average R^2 values of 0.930, 0.899, and 0.940 for buckwheat, clover, and orange blossom, respectively, using the PLS-1 method with first derivative data treatment. The optimal calibration method for the determination of adulterants in honey samples will be selected based on the highest R^2, lowest SEP, and lower validation error. Although corn syrup is a complex mixture of sugars, the PLS methods with first derivative data treatment predicted the quantity of added corn syrup to honey with a SEP of 1.99% to 2.22% (Table 5). Results indicate that reasonable prediction can be obtained on the 3 different varieties tested.

Data from the calibration sets of all 3 varieties of honey were merged, and new calibrations were obtained to develop a single calibration model to determine corn-syrup concentration in honey. The values of SEP, R^2, and the optimum number of factors used in the calibration method are shown in Table 6. The scatter plot (R^2 = 0.876) using PLS-1 with first derivative transformation is shown in Figure 3. The values of

Table 5—Calibration and validation results for different calibration methods using mean centering data processing for corn syrup in 3 varieties of honey

Honey type/ calibration method	Calibration			Validation	
	Factors[a]	R^{2b}	SEP[c]	R^{2b}	SEP[c]
Buckwheat					
PLS	8	0.976	10.78	0.920	2.188
PLS-first derivative	6	0.976	1.065	0.930	2.044
PCR	11	0.973	1.146	0.920	2.099
PCR-first derivative	9	0.979	1.077	0.928	2.005
Clover					
PLS	13	0.983	0.768	0.893	2.328
PLS-first derivative	12	0.980	0.693	0.899	1.993
PCR	20	0.980	0.693	0.899	1.993
PCR-first derivative	22	0.972	0.888	0.886	2.247
Orange Blossom					
PLS	8	0.934	1.708	0.920	2.366
PLS-first derivative	6	0.953	1.442	0.940	2.221
PCR	14	0.939	1.470	0.933	2.060
PCR-first derivative	9	0.951	1.810	0.943	2.123

a Optimum number of factors
b Correlation coefficient
c Standard error of prediction (%, w/w)

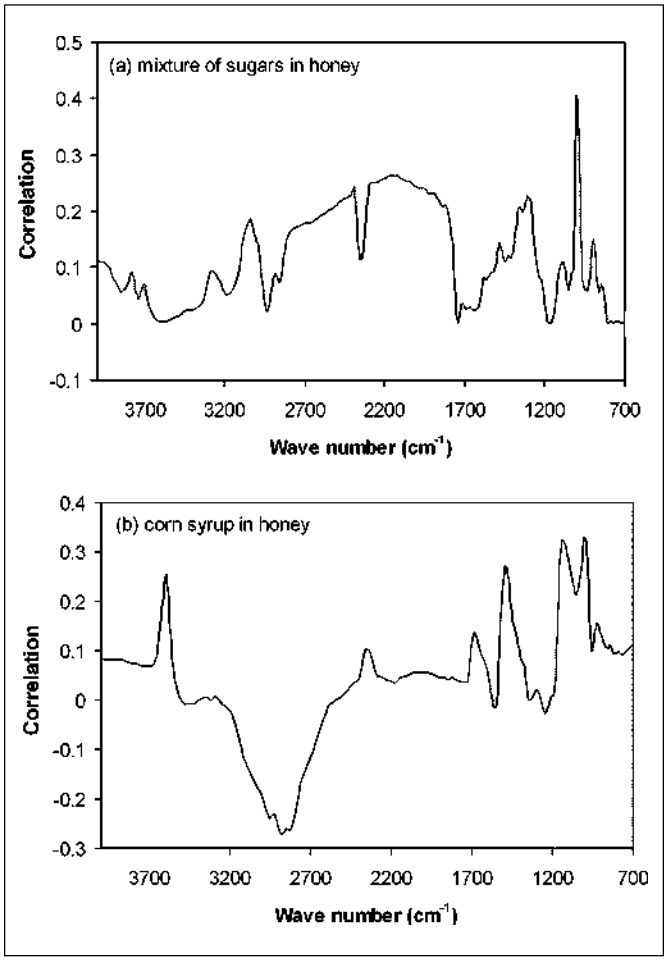

Figure 2—Correlation spectrum for (a) mixture of sugars and (b) corn syrup in honey

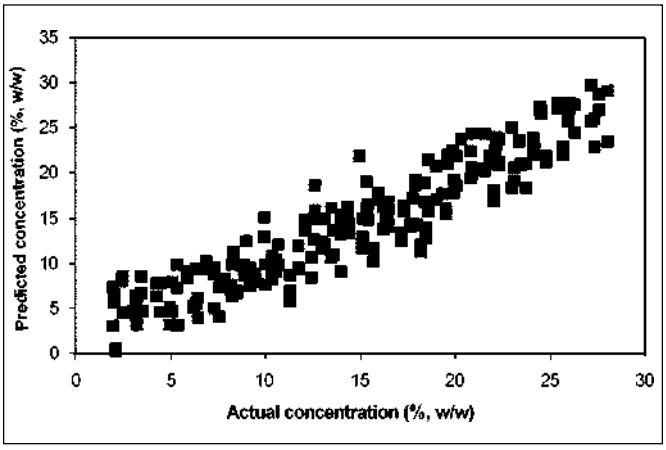

Figure 3—Scatter plot for corn syrup using PLS method with first derivative data transformation using the combined calibration data for 3 different varieties of honey

Table 6—Calibration and validation parameters for corn syrup using the combined calibration data for 3 different varieties of honey

Honey type/ calibration method	Calibration			Validation	
	Factors[a]	R^{2b}	SEP[c]	R^{2b}	SEP[c]
PLS	15	0.879	2.402	0.824	4.450
PLS-first derivative	10	0.876	2.512	0.758	5.482
PCR	18	0.859	2.593	0.741	5.559
PCR-first derivative	18	0.879	2.397	0.745	5.540

a Optimum number of factors
b Correlation coefficient
c Standard error of prediction (%, w/w)

the correlation coefficient were less compared to individual calibration models (Table 6). The developed models were used for further validation studies. The SEP values for calibration and validation sets were higher compared to those obtained for the individual varieties. Results show that although the combined model had a lower correlation yet, the potential for a unified model exists perhaps for selective groups.

Conclusions

A RAPID, INEXPENSIVE, AND NONCHEMICAL SPECTROSCOPIC method is proposed to be used instead of the current carbon isotope ratio analysis for the quantitative determination of sugar adulteration in honey samples. FTIR-ATR spectroscopy was used to estimate the added concentrations of corn syrup in 3 different varieties of honey samples. The added corn-syrup content in honey samples was determined by employing the PLS method with first derivative data transformation. The developed FTIR-ATR spectroscopy method is an effective analytical tool requiring no sample preparation, unlike the conventional chromatography methods. The precision of this method could be improved by studying larger and multicomponent sugar-mixture data sets. Prediction results indicate that MIR spectroscopy coupled with a suitable analytical procedure can be successfully used to detect sugar addition to honey and other related products. Further work must be conducted and include honey of many origins and with other adulterants.

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