Major Components of Honey Analysis by Near-Infrared Transflectance Spectroscopy

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NIR transflectance spectroscopy was used to analyze fructose, glucose, and moisture in honey. A total of 161 honey samples were collected during 1992 (46), 1995 (58), and 1996 (57). Samples were analyzed by instrumental, enzymatic (fructose and glucose), and refractometric (moisture) methods. Initially, different calibrations were performed for each of the 3 years of sampling. Good predictions were obtained for all three components with equations of the particular year. But good predictions were not always obtained when the equations calculated one year were applied to samples from another year. To perform a lasting calibration, unique calibration (121 samples) and validation (40 samples) sets were built; honeys of the 3 years were included in both sets. Good statistics (bias, standard error of validation (SEV), and R^2) were obtained for all three components of the validation set. No statistically significant differences ($p = 0.05$) were found between instrumental and reference methods.

Keywords: *NIR transflectance spectroscopy; honey analysis; fructose; glucose; moisture*

INTRODUCTION

The near-infrared (NIR) region was traditionally avoided by spectroscopists because of the difficulty in interpreting overlapping bands, because acute peaks do not appear in this region of the spectrum, and because its sensitivity is 10−100 times less than that of middle infrared (Osborne et al., 1993). However, in the past decades this technique has become more commonly used because of a range of factors such as the great advance in data processing technologies and computer programming, which made possible the treatment of the spectral information, the development of multivariate analysis techniques (Martens and Naes, 1993), and advances in the construction of spectrophotometers.

Determining the major components of foodstuff by classical analytical methods is slow and expensive, and requires highly qualified staff; therefore, the present chemical methods are not effective enough to meet the growing demands and offer low-cost solutions that are required. Near-infrared spectroscopy, which presents smaller absorption bands in at least 1 order of magnitude for each successive overtone, allows the use of more concentrated samples and longer optical paths than those used in middle-infrared spectroscopy. The main advantages of near-infrared spectroscopy for food analysis lie in its speed, the absence of or reduced need for sample pretreatment, and the absence of the use of chemicals (Osborne et al., 1993; Rodríguez-Otero et al., 1997).

Sugars are the principal constituents of honey, which, aside from determining its nutritious and energetic value, influence some of its important physical characteristics such as crystallization, hygroscopicity, and viscosity (Sabatini et al., 1989). Fructose and glucose together account for 85−95% of honey carbohydrates (Crane, 1976).

The amount of water in honey is of major importance to its stability against fermentation and granulation (White, 1978).

In the reviewed literature few articles were found that described honey analysis by NIR spectroscopy (Cho and Hong, 1998; Ha et al., 1998; Qiu et al., 1999).

The aim of our work was to analyze the major components of honey (fructose, glucose, and moisture), through NIR transflectance spectroscopy without the need for sample treatment. NIR transflectance spectroscopy analysis, a combination of reflectance and transmission measurement, provides a more reliable measure of absorbance of light scattering samples than do transmission techniques, because in transmission techniques the backscattered radiation is not measured, and in transflectance the radiation reflected before the ceramic is reached is also collected, so that all radiation not absorbed is measured (Osborne et al., 1993). Therefore, the NIR transflectance spectroscopy technique is appropriate for the analysis of pastelike products, such as honey.

EXPERIMENTAL PROCEDURES

Samples. A total of 161 different honeys were sampled in Galicia (NW Spain): 46 in 1992, 58 in 1995, and 57 in 1996 (Table 1). Moreover, from the annual sets of samples (with the total of 161 samples), two sets of honey samples were built, one calibration set of 121 samples and one validation set of 40 samples; in both sets samples from all 3 years were included. All samples bore the label "Producto Galego de Calidade-Mel de Galicia" (Diario Oficial de Galicia, 1989),

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Table 1. Fructose, Glucose, and Moisture: Mean and Standard Deviation in Honey Batches

		fructose		glucose		moisture	
batch	samples	mean	SD	mean	SD	mean	SD
1992	46	36.8	2.13	25.2	3.49	16.6	0.86
1995	58	36.0	2.48	23.4	3.34	17.0	0.91
1996	57	37.5	2.03	25.3	2.70	17.4	0.88

which guaranteed their origin. Before analysis was performed, the samples were warmed in a water bath to a maximum of 50 °C with the aim of melting the sugar crystals.

Reference Analyses. To determine fructose and glucose contents enzymatic analyses were performed (Boehringer-Mannheim GmbH, 1995) using a Kontron-Uvikon 922 A UV−vis double-beam spectrophotometer.

Moisture content was analyzed following the method described by White (1969). Moisture content was measured using a refractometer Atago Rx-5000.

NIR Analysis. A wavelength scanning instrument, NIR-Systems 6500, with a scanning range from 400 to 2500 nm and wavelength increments of 2 nm was used. Instrument checks recommended by the manufacturer were performed daily prior to use.

During the same week as reference analysis, samples were analyzed at room temperature (about 20 °C), in a 0.2 mm thick transflectance cell; approximately 1.5 g of honey was needed. Transflectance measurements of monochromatic light were made from 1108 to 2492 nm. The average of 25 spectral scans was taken for each sample; data were recorded as log 1/*R*, where *R* is the transflectance energy.

Statistics. ISI software was used for statistical analysis (ISI, 1992). Scatter correction was performed by standard normal variate transformation (SNV) and detrend method (Barnes et al., 1989) and by multiplicative scatter corretion (MSC) (Geladi et al., 1985).

A general Mahalanobis distance ("H" statistic) was calculated from principal components analysis (PCA) scores, and the *H* values were standardized by dividing them by the average *H* value for the calibration file. If a new sample spectra was more than 3.0 standardized units from the mean spectra of the calibration file, the sample was defined as a global *H* outlier and may not have accurate predictions.

The calibrations were performed by multiple linear regression (MLR), principal components regression (PCR), and modified partial least-square (MPLS) regression (Martens and Naes, 1993) using first and second derivatives of the spectra (Meuret et al., 1993). First derivative was calculated using a subtraction gap and smoothing segment of 4 data points (1,4,4). Second derivative was calculated using a subtraction gap and smoothing segment of 6 data points (2,6,6).

The optimum number of terms for the calibration that minimized overfitting was based on the standard error of cross validation (SECV). The approach used was as follows: 80% of the samples from the calibration set were used for calibration, and in the remaining 20% standard error of prediction (SEP) was calculated. This operation was carried out a total of five times; each time a different group for calibration and prediction was used. The SECV was calculated as the square root of the average of the squares of the five SEP values. The final calibration equation was developed with the total samples of the calibration set using the number of factors with the lowest SECV.

The critical *T* value for eliminating outliers was fixed at 2.5 (T = residual/SEC).

To check the performed calibration, the validation set, in which no samples of the calibration set were included, was used. The standard error of validation (SEV) and R^2 of reference versus NIR values were calculated.

RESULTS AND DISCUSSION

Figure 1 shows the mean spectrum of all 161 honeys. The following bands can be observed: at 1450 nm the first overtone of the O−H stretch, at 1765 nm the first overtone of the CH_2 group, at 1940 nm the combination O−H stretch and bend band, at 2100 nm the combination of O−H deformation band and C−O stretch band, at 2280 nm the combination of C−H stretch and deformation band, and finally, at 2345 nm the combination of CH_2 stretch and deformation band (Osborne et al., 1993).

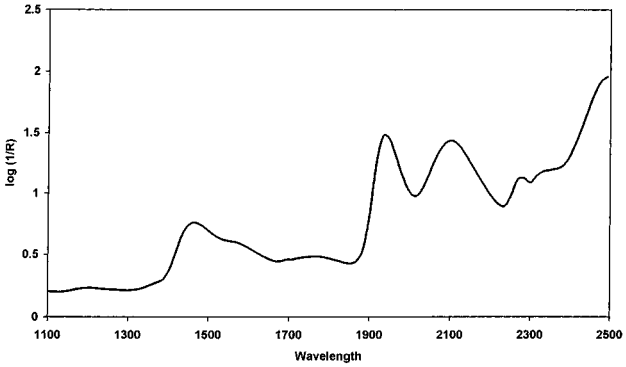

Figure 1. Mean spectrum of honeys.

Table 2. Repeatability of Fructose (Enzymatic), Glucose (Enzymatic), and Moisture (Refractometric) Analysis by Reference and NIR Methods

method	fructose	glucose	moisture
reference	0.074	0.052	0.049
NIR	0.250	0.274	0.051

Repeatability. Repeatability of the standard deviation (S_r) (Table 2) of enzymatic methods (fructose and glucose), the refractometric method (moisture), and NIR transflectance spectroscopy were calculated over 11 duplicated analyses (Miller and Miller, 1989). With the objective of considering the errors of sample packaging, for NIR determination samples were analyzed after each duplicate was repackaged.

Regarding moisture, a very low S_r value was found by NIR transflectance spectroscopy; S_r was almost the same as that determined by the refractometric method. NIR transflectance spectroscopy yielded very similar S_r values for the sugars, fructose, and glucose, and higher values than those found by enzymatic methods. However, repeatability values of NIR spectroscopy analysis, despite being higher than those of enzymatic method, are acceptable for a rapid method.

Calibration. To evaluate the different calibrations, SEC, SECV, R^2 of the calibration sets and bias, SEV, and R^2 of the validation sets were considered.

MPLS achieved in all cases the best calibration equations. Low differences were found for statistical results of calibrations when SNV and detrend or MSC were used for scatter correction of radiation. Therefore, SNV and detrend were chosen for all calibrations, with the aim of simplifying the discussion. For most of the cases first derivative was chosen, second derivative being preferred only for two calibrations.

Initially, different calibrations were performed for each of the three sample sets, which correspond to years 1992, 1995, and 1996. Statistical data were acceptable for all three components analyzed (Table 3). SECV values were only slightly higher than those of SEC; this means that no overfitting occurred. Therefore, a good calibration can be achieved for all three components each year. However, when the equations obtained for each of those years were applied to the samples of the

Table 3. Statistical Data for 1992, 1995, and 1996 Calibration Sets

component	samples	PLS terms	SEC	SECV	R^2	derivative
		1992 Honeys				
fructose	45	6	0.51	0.60	0.94	first
glucose	44	6	0.51	0.60	0.98	first
moisture	44	3	0.12	0.15	0.98	first
		1995 Honeys				
fructose	56	7	0.35	0.45	0.98	first
glucose	55	6	0.38	0.50	0.99	first
moisture	54	7	0.08	0.15	0.99	first
		1996 Honeys				
fructose	53	7	0.25	0.36	0.99	second
glucose	54	5	0.68	0.83	0.93	second
moisture	53	4	0.08	0.09	0.99	first

Table 4. Annual Calibrations: Statistical Data of Validations

equation	component	samples	bias	SEV	R^2
		Validation with 1995 Honeys			
1992	fructose	58	0.89	1.21	0.89
	glucose	58	−0.09	1.07	0.90
	moisture	58	0.06	0.21	0.96
		Validation with 1996 Honeys			
	fructose	57	0.84	1.02	0.92
	glucose	57	0.39	0.94	0.91
	moisture	57	−0.04	0.21	0.95
		Validation with 1992 Honeys			
1995	fructose	46	−0.67	0.90	0.92
	glucose	46	0.73	1.04	0.95
	moisture	46	−0.11	0.21	0.96
		Validation with 1996 Honeys			
	fructose	57	0.07	0.56	0.92
	glucose	57	0.17	0.91	0.89
	moisture	57	−0.21	0.29	0.96
		Validation with 1992 Honeys			
1996	fructose	46	−0.60	0.86	0.92
	glucose	46	−0.76	0.91	0.93
	moisture	46	0.29	0.32	0.98
		Validation with 1995 Honeys			
	fructose	58	−0.67	0.69	0.92
	glucose	58	0.73	1.48	0.87
	moisture	58	0.21	0.24	0.96

Table 5. Average *H* (Outliers in Parentheses) for the Annual Calibrations

samples	equation 1992	equation 1995	equation 1996
1992	1.00	2.08 (8)	1.85 (4)
1995	1.74 (6)	1.00	2.07 (8)
1996	2.23 (15)	1.38 (5)	1.00

others years, the differences between SEC and SEV were higher, and in many cases SEV duplicated the value of SEC and the values of R^2 for the validation sets decreased in relation with the same parameter in calibration sets (Table 4). This can be attributable to the H outliers ($H > 3$) found in all sets when equations of different years were used (Table 5).

The most stable of the three components is moisture; B coefficients of wavelengths were smaller and less sharp for this component (Figures 2−4). Calibrations worked well from year to year in the prediction of this parameter, and the validation statistics were good in all cases ($R^2 \geq 0.95$ and the maximum value of SEV was 0.32). However, the prediction of sugars composition was not as accurate: for fructose, R^2 was within 0.89 and 0.92; for glucose, R^2 was within 0.87 and 0.95. The SEV values were much higher than those of SEC and SECV for all three components and bias values were also high in most of the cases.

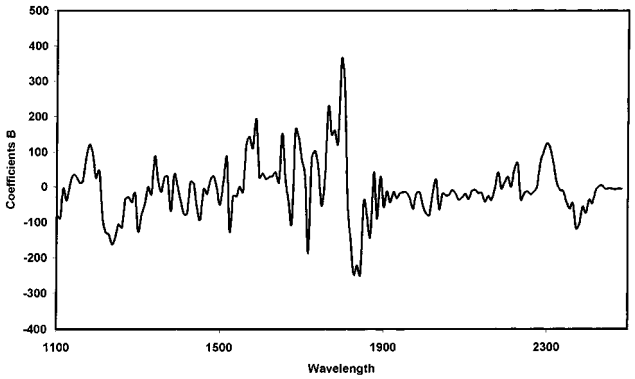

Figure 2. Coefficients *B* of fructose equation.

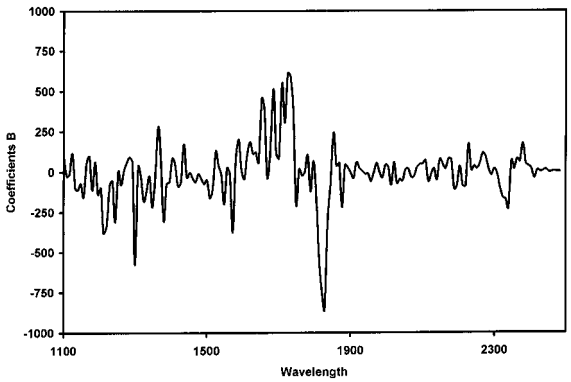

Figure 3. Coefficients *B* of glucose equation.

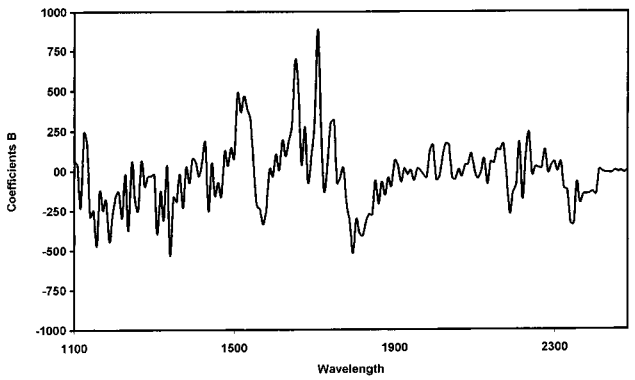

Figure 4. Coefficients *B* of moisture equation.

Good predictions were achieved for all three components with equations for the particular year, but predictions were not always accurate when the equations calculated for one year were applied to samples from an other year.

A complete annual calibration can be a serious hurdle for the application of this technique in honey analysis. Because instrument calibration is cumbersome work, which requires skilled staff to carry out the reference analytical methods, the calibration was not justified unless a large number of samples were to be analyzed in routine.

With the aim of achieving a lasting calibration and overcoming that inconvenience, unique calibration and validation sets were built. The initial 161 samples were split in two sets: one was used to perform the calibration, containing 121 samples; another set of 40 samples was used to validate the obtained calibration. Honeys of the 3 years were included in both sets of samples. If this calibration was accurate, only a few samples should

Table 6. Statistical Data for Global Calibration Set

component	samples	PLS terms	SEC	SECV	R^2	derivative
fructose	117	7	0.44	0.48	0.96	first
glucose	117	9	0.60	0.71	0.97	first
moisture	118	7	0.12	0.14	0.98	first

Table 7. Global Calibration: Statistical Data of Validation

component	samples	bias	SEV	R^2
fructose	40	0.02	0.35	0.98
glucose	40	0.05	0.70	0.95
moisture	40	−0.03	0.17	0.96

be added each year to extend the calibration and keep it effective for new harvests.

For the global calibration obtained with the 121 samples of the calibration set, SEC and R^2 where similar to those of the averages of annual calibrations (Table 6). When these calibrations were validated with the 40 samples of the validation set, the average H value was 1.19 and only two H outliers were found. SEV, bias, and R^2 (Table 7) were always much better than when equations from one year were applied to the honeys of another year.

The number of PLS terms is not high for the number of samples of the global calibration set. When the differences between SEC and SEV and between R^2 for calibration and validation sets are small, overfitting is avoided. Although SEV values are not often lower than SEC values, similar cases can be found in the literature in which calibration sets are wide enough and representative, and strong calibrations are achieved (Blattner et al., 1985; Frankhuizen and van der Veen, 1985; Pierce and Wehling, 1994; Katayama et al., 1996; Albanell et al. 1999; Laporte and Paquin 1999).

Our results were better than those of Cho and Hong (1998) and Ha et al. (1998) and similar to those of Qui et al. (1999).

To compare the results obtained by NIR spectroscopy with those obtained by the reference methods, for all three components of the validation set, linear regression, and paired t test were applied (Miller and Miller, 1989). When the slope and intercept of NIR values were calculated versus reference values, no statistical differences ($p = 0.05$) were found from the theoretical values 1.00 and 0.00, respectively. The calculated t values were less than the theoretical t values ($p = 0.05$). Thus, the null hypothesis was retained: the methods do not give significantly different results. Graphical comparisons between reference values and NIR predicted values for the global validation set are shown in Figures 5−7.

Conclusions. NIR transflectance spectroscopy is an adequate technique for analysis of major components in honey, without any sample pretreatment. Repeatability values of NIR spectroscopy are acceptable for a rapid method. Good annual calibration can be obtained for all three components. However, when the calibration equation of 1 year is applied to samples of another year, good predictions are not always performed, and stable calibration was only achieved for moisture. To achieve a lasting calibration, new samples should be included each year to ensure the accuracy of the predictions of fructose, glucose, and moisture contents. MPLS regression produced the best calibrations.

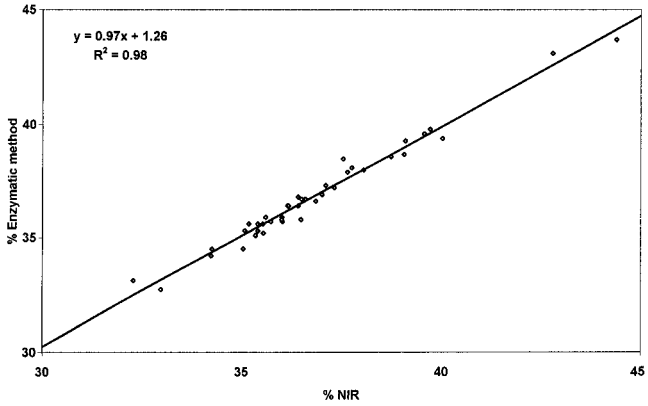

Figure 5. Validation set: fructose content. NIR vs enzymatic method.

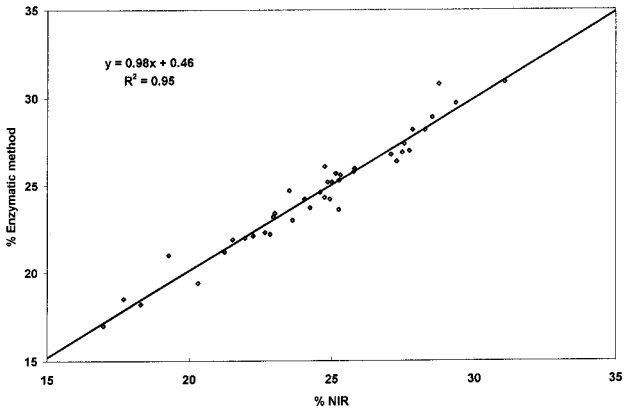

Figure 6. Validation set: glucose content. NIR vs enzymatic method.

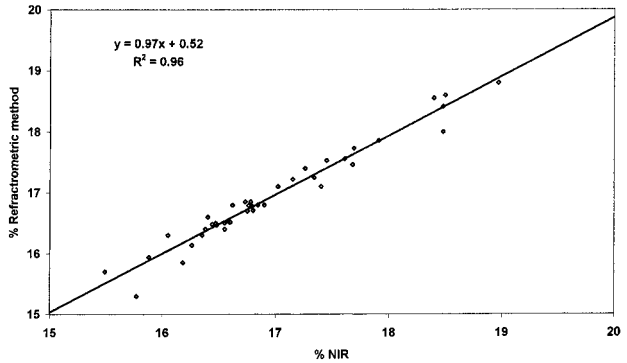

Figure 7. Validation set: moisture content. NIR vs refractometric method.

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