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Determination of Polarimetric Parameters of Honey by Near-Infrared Transflectance Spectroscopy

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NIR transflectance spectroscopy was used to determine polarimetric parameters (direct polarization, polarization after inversion, specific rotation in dry matter, and polarization due to nonmonosaccharides) and sucrose in honey. In total, 156 honey samples were collected during 1992 (45 samples), 1995 (56 samples), and 1996 (55 samples). Samples were analyzed by NIR spectroscopy and polarimetric methods. Calibration (118 samples) and validation (38 samples) sets were made up; honeys from the three years were included in both sets. Calibrations were performed by modified partial least-squares regression and scatter correction by standard normal variation and detrend methods. For direct polarization, polarization after inversion, specific rotation in dry matter, and polarization due to nonmonosaccharides, good statistics (bias, SEV, and R^2) were obtained for the validation set, and no statistically ($p = 0.05$) significant differences were found between instrumental and polarimetric methods for these parameters. Statistical data for sucrose were not as good as those of the other parameters. Therefore, NIR spectroscopy is not an effective method for quantitative analysis of sucrose in these honey samples. However, NIR spectroscopy may be an acceptable method for semiquantitative evaluation of sucrose for honeys, such as those in our study, containing up to 3% of sucrose. Further work is necessary to validate the uncertainty at higher levels.

KEYWORDS: NIR spectroscopy; honey analysis; polarimetric parameters; sucrose

INTRODUCTION

More than 95% of honey solids are carbohydrates; they are mainly simple sugars or monosaccharides. In nearly all honey types fructose predominates; a few honeys appear to contain more glucose than fructose. These two sugars together account for 85−95% of honey carbohydrates. More complex sugars (oligosaccharides), made up of two or more molecules of glucose and fructose, constitute the remainder, except for a trace of polysaccharide (*1*, *2*). Research has shown the presence of at least eleven disaccharides (maltose, kojibiose, turanose, isomaltose, sucrose, maltulose, nigerose, α,*β*-trehalose, gentiobiose, and laminaribiose), at least ten trisaccharides (erlose, theanderose, panose, maltotriose, 1-kestose, isomaltotriose, melizitose, isopanose, centose, and 3-α-isomaltosylglucose), and at least two higher oligosaccharides (isomaltotetraose and isomaltopentaose) (*3*).

Among many other substances of natural origin, honey has the property of rotating the polarization plane of polarized light. This is one further property that depends largely on types and relative proportions of the sugars in honey. Because each sugar has a specific and consistent effect, and the total optical rotation is dependent on concentration, early analysts used optical rotation under various specified conditions as a means of sugar analysis. A generalization that appears to remain valid is that floral honeys are levorotatory, and honeydew or adulterated honeys are usually dextrorotatory. This is a consequence of the normal preponderance in floral honey of fructose, which has a negative specific rotation ($[\alpha]_D^{20} = -92.4°$), over that of glucose ($[\alpha]_D^{20} = +52.7°$). Honeydew types are usually somewhat lower in fructose content and contain melezitose ($[\alpha]_D^{20} = +88.2°$) or erlose ($[\alpha]_D^{25} = +121.8°$) (*4*) which, together with glucose, usually give a positive net optical rotation (*1*, *5*). Although the conventional boundary between the two types has been considered to be 0°S (*2*), White (*6*) has proposed that conditions of symmetry argue for a −2°S boundary.

The foremost polarimetric parameters of honey are direct polarization, which gives a global idea of the sugars present in the sample, and polarization after inversion; with both param-

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eters sucrose may be calculated (*7*). The limits for apparent sucrose content in the Codex Alimentarius (*8*) and in the European Regulations (*9*) is 5% (w/w) for floral honeys and 10% (w/w) for honeydew honey, blends of honeydew honey and Blossom honey, Robinia, Lavander, and *Bankesia menziessii* honeys.

The main advantages of near-infrared spectroscopy for food analysis are its speed, the absence of (or reduction in) sample pretreatment, and the avoidance of chemical use (*10*, *11*). In the literature reviewed, few articles were found that described honey analysis by NIR spectroscopy (*12*−*15*) and no polarimetric methods were studied in any of them.

The aim of our work was to determine the polarimetric parameters of honey by NIR transflectance spectroscopy, avoiding sample pretreatment. Direct polarization, polarization after inversion, specific rotation in dry matter, polarization due to nonmonosaccharides, and sucrose content were dealt with in this study.

EXPERIMENTAL PROCEDURES

Samples. In total, 156 different floral honeys were sampled in Galicia (NW Spain): 45 in 1992, 56 in 1995, and 55 in 1996. All samples bore the label "Producto Galego de Calidade-Mel de Galicia" (*16*), which guarantees 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.

Polarimetric Determinations. Polarimetric readings were performed by a Perkin-Elmer 241 polarimeter, fitted with a sodium lamp and with a microcell of 100 mm length, which was thermoregulated at 20 °C by use of a Selecta Ultraterm water bath. This equipment is able to measure the angular rotation to an accuracy of 0.001 circular degrees.

Direct polarization was determined following the method of Bogdanov et al. (*17*), in samples of 12.000 g of honey/100 mL clarified with Carrez solutions and maintained during 24 h to avoid mutarotation effect. In this work, unlike Bogdanov et al. (*17*), the corresponding readings were carried out, in accordance with the recommendations of the AOAC (*7*), in a 100 mm tube, because the filtered solution shows color.

Inversion was performed, according to the general procedure of Walker (*18*), on a portion of the solution clarified as described above. The reading of polarization after inversion was carried out after 24 h to avoid mutarotation effect. The result of this reading referred to a concentration of 12.000 g of honey/100 mL.

To convert the results obtained in this work with the different saccharimeter scales, it is necessary to consider the tube length, the normal weights, and the conversion factors of the different saccharimeter scales (*7*).

Moisture, glucose, and fructose contents were determined in a previous work (*15*). Specific rotation $[\alpha]_D^{20}$ in dry matter was calculated according to Bogdanov et al. (*17*), using the corresponding values of direct polarization and moisture. Note that the specific rotation is the angle of rotation of polarized light at the wavelength of the sodium D line at 20 °C of an aqueous solution of 1 dm depth and containing 1 g/mL of the sample (*17*). Polarization due to nonmonosaccharides, dealt with for the first time in this work, was calculated by subtracting glucose and fructose polarization values of 12.000 g of honey/100 mL in a 100 mm tube from the determined direct polarization values. Sucrose was calculated from the difference between direct polarization and polarization after inversion (*7*).

NIR Analysis. A wavelength-scanning instrument, NIRSystems 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.

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.

Table 1. Direct Polarization, Polarization after Inversion, Specific Rotation in Dry Matter, Polarization Due to Nonmonosaccharides, and Sucrose in Calibration and Validation Sets (mean and standard deviation)

	calibration set		validation set	
parameter	mean	S_d	mean	S_d
direct polarization (degrees)	–0.810	0.647	–0.766	0.563
polarization (degrees) after inversion	–0.966	0.668	–0.920	0.564
specific rotation (degrees mL g^{-1} dm^{-1}) in dry matter	–8.187	6.574	–7.701	5.693
polarization (degrees) due to nonmonosaccharides	1.729	0.512	1.748	0.534
sucrose (% w/w)	1.46	0.51	1.48	0.36

Table 2. Statistical Data for Calibration and Validation Sets

Calibration Set

parameter	samples	PLS terms	SEC	SECV	R^2
direct polarization (degrees)	118	10	0.037	0.049	0.997
polarization (degrees) after inversion	118	8	0.059	0.068	0.992
specific rotation (degrees mL g^{-1} dm^{-1}) in dry matter	118	10	0.380	0.540	0.996
polarization (degrees) due to nonmonosaccharides	118	8	0.087	0.113	0.977
sucrose (% w/w)	118	8	0.32	0.38	0.612

Validation Set

parameter	samples	bias	SEV	R^2
direct polarization (degrees)	38	–0.009	0.037	0.996
polarization (degrees) after inversion	38	–0.004	0.051	0.992
specific rotation (degrees mL g^{-1} dm^{-1}) in dry matter	38	–0.086	0.392	0.996
polarization (degrees) due to nonmonosaccharides	38	–0.008	0.070	0.984
sucrose (% w/w)	38	0.014	0.25	0.336

Statistics. ISI software was used (*19*). Scatter correction was performed by standard normal variate transformation (SNV) and detrend method (*20*), and by multiplicative scatter correction (MSC) (*21*).

A general Mahalanobis distance ("H" statistic) was calculated from principal component analysis (PCA) scores, and the *H* values were standardized by dividing them by the average *H* value for the calibration file. If a new spectra sample 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 give accurate predictions.

The calibrations were performed by modified partial least-squares (MPLS) regression (*22*) using first and second derivatives of the spectra (*23*). The first derivative was calculated using a subtraction gap and smoothing segment of 4 data points (1, 4, 4). The 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 minimizing 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 5 times, each time using a different group for calibration and prediction. The SECV was calculated as the square root of the average of the squares of the 5 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.

Standard error of calibration (SEC) was calculated, and the critical *T* value for eliminating outliers was set at 2.5 (T = residual/SEC).

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

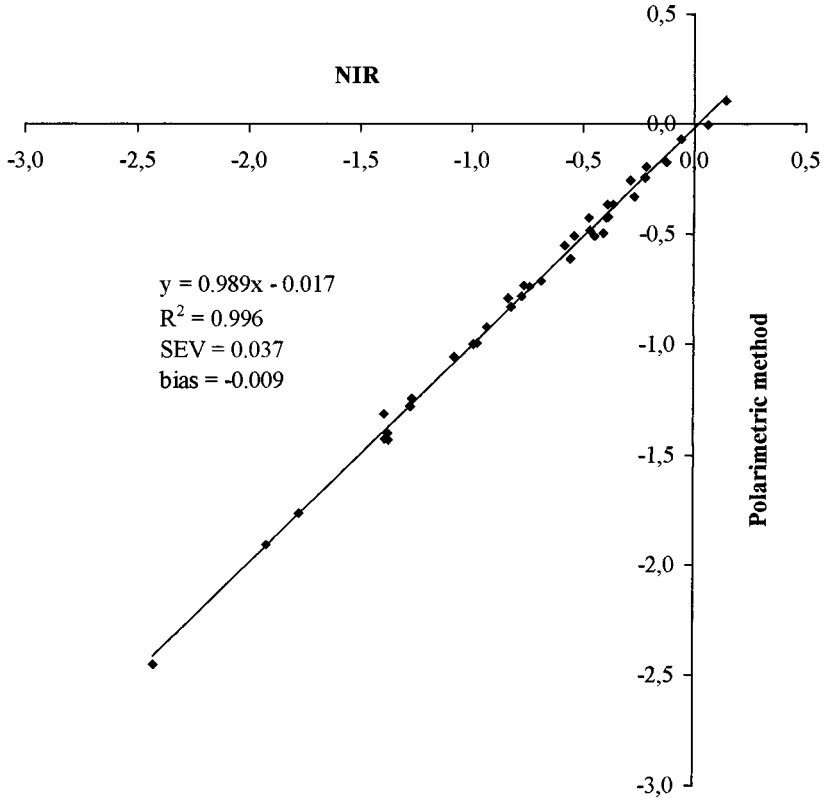

Figure 1. Direct polarization (degrees). NIR versus polarimetric method (validation set).

RESULTS AND DISCUSSION

The mean NIR transflectance spectrum of honeys belonging to calibration and validation sets is shown in a previous work (*15*).

Initially, different calibrations were tested for each of the three sample sets, which correspond to years 1992, 1995, and 1996. Statistical data from these calibrations were acceptable for direct polarization, polarization after inversion, specific rotation in dry matter, and polarization due to nonmonosaccharides. However, when the equations obtained for each of those years were validated with the samples from the other years, the differences between SEC and SEV were high: in many cases SEV duplicated the value of SEC or even more. In a previous work (*15*) similar results were obtained for glucose, fructose, and moisture. This may be attributable to the "H" outliers ($H > 3$) found in all sets when equations of different years were used. As a result, good predictions were achieved for all three components with equations from the same year, but this may not be the case always when the equations calculated for one year are applied to samples from another year. But a complete annual calibration can be a serious hurdle to the application of this technique in honey analysis, considering the fact that instrument calibration is a cumbersome task, and skilled staff are required to carry out the reference analytical methods. Therefore, the calibration is not justified unless large numbers of samples are to be analyzed routinely.

With the aim of achieving a lasting calibration and overcoming that problem, unique calibration and validation sets were made up: the initial 156 samples were split into two sets, one to perform the calibration, containing 118 samples; and another, containing 38 samples, to validate the obtained calibration. Honeys from the three years were included in both sets of samples. Calibration and validation sets were randomly selected, with the sole condition that the samples with minimum and maximum values for each parameter be included in the calibration set, with the aim of avoiding extrapolation. Two *H* outliers were removed from calibration set. Mean and standard deviation (S_d) of direct polarization, polarization after inversion, specific rotation in dry matter, polarization due to nonmonosaccharides and sucrose of the calibration and validation sets are shown in Table 1. If this calibration is accurate, only a few samples should be added each year to extend the calibration and keep it effective for new harvests.

To assess the results obtained and the suitability of NIR transflectance spectroscopy, SEC, SECV, and the R^2 values for the calibration set (Table 2), and bias, SEV, R^2 (Figures 1−5), and mean square prediction error (MSPE) (Table 3) for the validation set were evaluated.

Relatively small differences were found for the statistical results of calibrations when comparing the use of SNV and detrend or MSC for scatter correction of radiation. Therefore, SNV and detrend was chosen with the aim of simplifying the discussion of the results. Also, small differences were found between using first or second derivative of spectra; therefore, in addition to simplifying discussion, the better ratio of signal/noise for the lower order of the derivatives (*10*) recommends the use of first derivative.

Good statistics were obtained for direct polarization, polarization after inversion, specific rotation in dry matter, and polarization due to nonmonosaccharides. The number of PLS terms was not high for the number of samples in the calibration set: less than one per 10 samples of the calibration set (Table 2). Consequently, no overfitting should be expected, which was confirmed by the low differences found between SEC and SECV of the calibration set. High values of R^2 were obtained, over

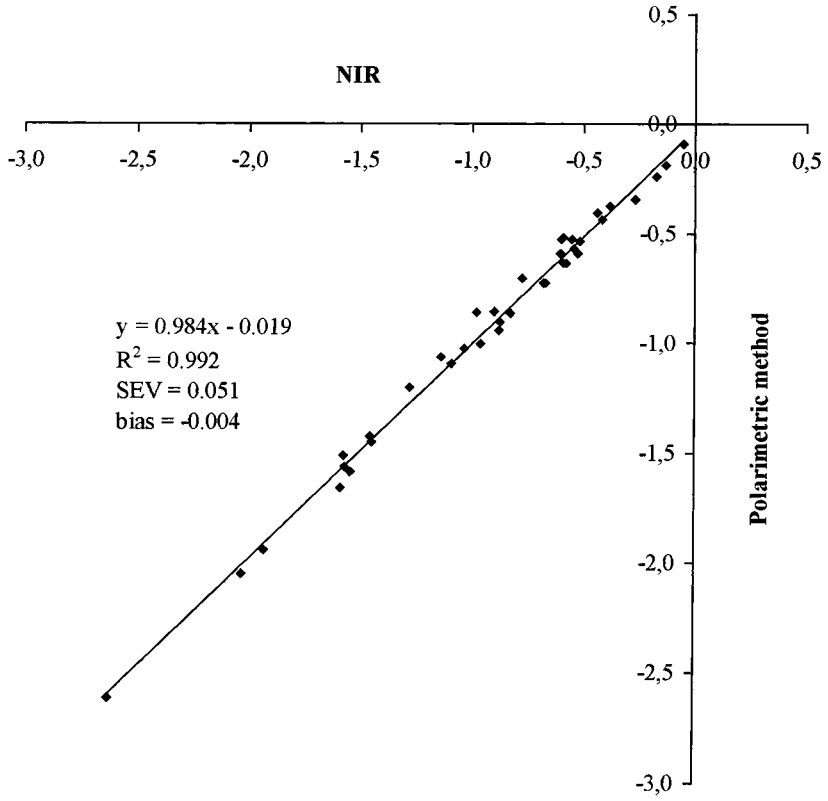

Figure 2. Polarization (degrees) after inversion. NIR versus polarimetric method (validation set).

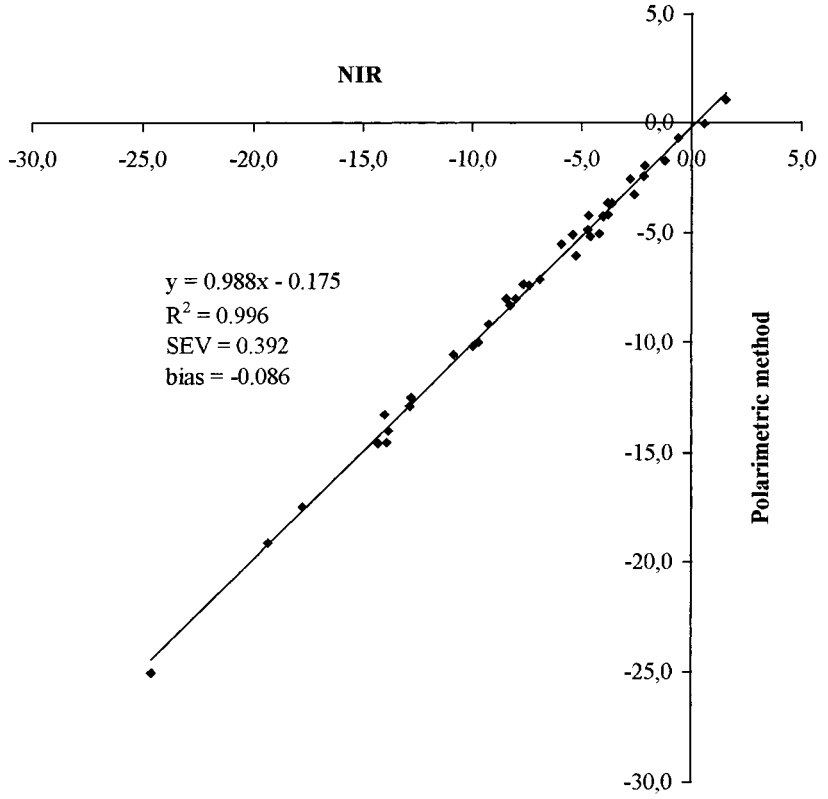

Figure 3. Specific rotation (degrees mL g^{-1} dm^{-1}) in dry matter. NIR versus polarimetric method (validation set).

0.99 for direct polarization, polarization after inversion, and specific rotation in dry matter, and 0.977 was obtained for polarization due to nonmonosaccharides. When the equations of calibration were applied to the validation set for direct polarization, polarization after inversion, specific rotation in dry matter, and polarization due to nonmonosaccharides, low values of bias were found; moreover, similar values between SEC and SEV and high values of R^2 were observed.

To compare the results obtained by NIR spectroscopy for direct polarization, polarization after inversion, specific rotation

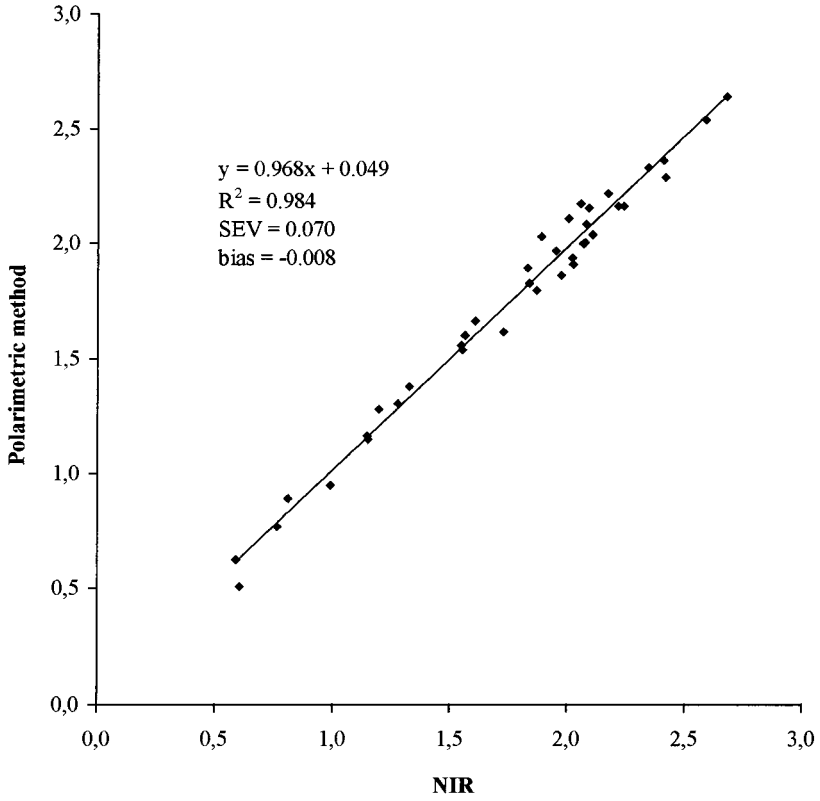

Figure 4. Polarization (degrees) due to nonmonosaccharides. NIR versus polarimetric method (validation set).

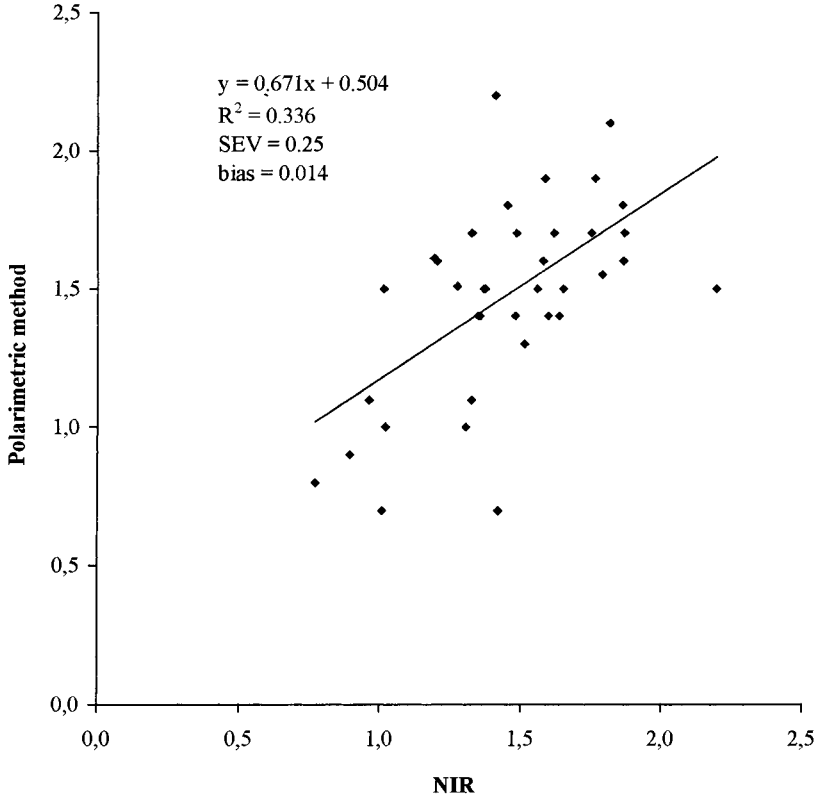

Figure 5. Sucrose (% w/w). NIR versus polarimetric method (validation set).

in dry matter, and polarization due to nonmonosaccharides of the validation set with those obtained by the polarimetric methods, linear regression and paired "*t*" test were applied (*24*): (i) When calculating the slope and intercept of NIR values versus reference values, no statistical differences ($p = 0.05$) were found from the theoretical values of 1.00 and 0.00, respectively; (ii) the calculated "*t*" values were lower than the theoretical "*t*" values ($p = 0.05$).

Therefore, the null hypothesis was retained: the two methods, in general, did not give significantly different results. Graphic

Table 3. Mean Square Prediction Error and Its Components (percentage in parentheses)

parameter	MSPE	errors in central tendency	errors due to regression	unexplained error
direct polarization (degrees)	0.0014	7.9×10^{-5} (5.6%)	3.6×10^{-5} (2.5%)	1.3×10^{-3} (91.9%)
polarization (degrees) after inversion	0.0025	1.8×10^{-5} (0.7%)	8.3×10^{-5} (3.3%)	2.4×10^{-3} (96.0%)
specific rotation (degrees mL g^{-1} dm^{-1}) in dry matter	0.15	7.5×10^{-3} (4.9%)	4.3×10^{-3} (2.8%)	0.14 (92.3%)
polarization (degrees) due to nonmonosaccharides	0.0049	6.5×10^{-5} (1.3%)	3.1×10^{-4} (6.3%)	4.5×10^{-3} (92.4%)
sucrose (% w/w)	0.093	7.1×10^{-4} (0.8%)	1.0×10^{-2} (10.8%)	8.2×10^{-2} (88.4%)

comparisons between polarimetric values and NIR-predicted values of the validation set are shown in Figures 1−4.

The MSPE is the sum of three types of errors (*25*): errors in central tendency, errors due to regression, and errors due to uncontrolled disturbance or unexplained errors (Table 3). Errors in central tendency are also known as mean bias; this error is very small in percentage terms for the four parameters.

The errors linked to regression will be equal to zero when the slope of regression is unity. This error also accounts for a small proportion of the MSPE.

The unexplained error accounts for a high percentage of the MSPE: 88.4% for sucrose and more than 90% for the other four parameters.

In the case of sucrose, the statistical data (Tables 2 and Figure 5) were worse than those of the other parameters, the ratios of S_d/SEC for the calibration set and S_d/SEV for the validation set were 1.6 and 1.4, respectively, very low values for a quantitative determination. Therefore, R^2 values for both calibration and validation sets were also very low; and when the linear regression test was applied (*24*), the slope and intercept of NIR values versus reference values show statistical differences ($p = 0.05$) from the theoretical values of 1.00 and 0.00, respectively (Figure 5). As a result, it can be stated that NIR reflectance spectroscopy is not an effective method for quantitative analysis of sucrose in these honey samples. This fact may be due to the narrow range of variation of sucrose in the honey samples: 0.00−3.70% (w/w) and 0.70−2.20% (w/w) for the calibration and validation sets, respectively. Although direct polarization and polarization after inversion are well-predicted, when the sucrose was calculated from differences between direct polarization and polarization after inversion, statistical data for sucrose did not improve. This is also due to the narrow range of the differences between direct polarization and polarization after inversion.

However, NIR spectroscopy may be an acceptable method for semiquantitative evaluation of sucrose, with a confidence interval of ± 0.50% (w/w) for honeys such as those in our study, containing up to 3% (w/w) of sucrose. Further work is necessary to validate the uncertainty at higher levels. As most honey samples contain less than 3% (w/w) sucrose (*3*), the polarimetric analysis of sucrose could be avoided in a high percentage of samples, and honeys could be classified as correct or incorrect (*8*, *9*) by NIR spectroscopy.

CONCLUSIONS

NIR transflectance spectroscopy is an adequate technique for determination of direct polarization, polarization after inversion, specific rotation in dry matter, and polarization due to nonmonosaccharides in honey without any previous sample treatment. NIR transflectance spectroscopy is not a suitable technique for quantitative determination of sucrose, at least not in sets of samples with a low variation in this component. However, it is an acceptable technique for classifying most samples as correct or incorrect in accordance with the Codex Alimentarius (*8*) and the European Regulations (*9*).

After MSPE analysis it can be concluded that the main source of error is unexplained error, accounting for about 90% for all the parameters studied.

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LITERATURE CITED

(1) Crane, E. *Honey: A Comprehensive Survey*; Heinemann: London, U.K., 1976.
(2) Crane, E. *The Hive and the Honey Bee*. Dadant & Sons: Hamilton, IL, 1992.
(3) White, J. W. Jr. Honey. In *Advances in Food Research*; Chichester, C. O., Mrak, E. M., Stewart, G. F., Eds.; Academic Press: New York, 1978; Vol. 24, p 303.
(4) White, J. W. Jr., Maher, J. α-maltosyl β-D-fructofuranoside, a trisaccharide enzymically synthesized from sucrose. *J. Am. Chem. Soc*. **1953**, *75*, 1259−1260.
(5) Crane, E. *Bees and Beekeeping: Science, Practice and World Resources*. Heinemann Newnes: Oxford, U.K., 1990.
(6) White, J. W. Jr. Detection of honey adulteration by carbohydrate analysis. *J. Assoc. Off. Anal. Chem*. **1980**, *63*, 11−18.
(7) AOAC International. *Official Methods of Analysis*, 16th edition; 5th revision, version CD-ROM; AOAC International: Gaithersburg, MD, 1999.
(8) Codex Alimentarius Commission. 1969. Recommended European Standard for Honey; CAC/RS-12-1969, Jt, FAO/WHO Food Standards Program, Rome. Reprinted in *Bee World* **1970**, *51*, 79−91.
(9) European Economic Community. Council Directive of 22 July 1974 on the Harmonisation of the Laws of the Member States Relating to Honey (74/409/EEC). Official Journal of the European Communities of 12 August 1974, No. L221:10-14, Brussels, Belgium, 1974.
(10) Osborne, B. G.; Fearn, T.; Hindle, P. H. *Practical Near-Infrared Spectroscopy with Applications in Food and Beverage Analysis*; Longman Scientific & Technical: Harlow, U.K., 1993; pp 28−33.
(11) Rodríguez-Otero, J. L.; Hermida, M.; Centeno, J. Analysis of dairy products by near-infrared spectroscopy: a review. *J. Agric. Food Chem*. **1997**, *45*, 2815−2820.
(12) Cho, H. J.; Hong, S. H. Acacia honey quality measurement by near-infrared spectroscopy. *J. Near Infrared Spectrosc*. **1998**, *6*, A329-A331.

(13) Ha, J.; Koo, M.; Ok, H. Determination of the constituents of honey by near-infrared spectroscopy. *J. Near Infrared Spectrosc*. **1998**, *6*, A367−A369.
(14) Qiu, P. Y.; Ding, H. B.; Tang, Y. K.; Xu, R. J. Determination of chemical composition of commercial honeys by near-infrared spectroscopy. *J. Agric. Food Chem*. **1999**, *47*, 2760−2765.
(15) García-Alvarez, M.; Huidobro, J. F.; Hermida, M.; Rodríguez-Otero, J. L. Major components of honey analysis by near-infrared transflectance spectroscopy. *J. Agric. Food Chem*. **2000**, *48*, 5154−5158.
(16) Diario Oficial de Galicia. Orden del 8 de febrero de 1989 de la Xunta de Galicia por la que se aprueba el Reglamento de Denominación "Producto Galego de Calidade-Mel de Galicia"; Xunta de Galicia; Santiago de Compostela, Spain, 1989.
(17) Bogdanov, S.; Martin, P.; Lullmann, C. Harmonised methods of the European Honey Commission. *Apidologie* **1997**, 58−59.
(18) Walker, H. S. A simplified inversion process for the determination of sucrose by double polarisation. *J. Ind. Eng. Chem*. **1917**, *9*, 490.
(19) ISI Windows Near-Infrared Software. Intrasoft International: Brussels, Belgium, 1998.
(20) Barnes, R. J.; Dhanoa, M. S.; Lister, S. J. Standard normal variate transformation and detrending of near-infrared diffuse reflectance spectra. *Appl. Spectrosc*. **1989**, *43*, 772−777.
(21) Geladi, P.; McDougall, D.; Martens, H. Linearization and scatter correction for near-infrared reflectance spectra of meat. *Appl. Spectrosc.* **1985**, *39*, 491−500.
(22) Martens, H.; Naes, T. *Multivariate Calibration*; John Wiley & Sons: Chichester, U.K., 1993.
(23) Meuret, M.; Dardenne, P.; Biston, R.; Poty, O. The use of NIR in predicting nutritive value of Mediterranean tree and shrub foliage. *J. Near Infrared Spectrosc*. **1993**, *1*, 45−54.
(24) Miller, J. C.; Miller, J. N. *Statistics for Analytical Chemistry*; Ellis Horwood: Chichester, U.K., 1989.
(25) Dhanoa, M. S.; Lister, S. J.; France, J.; Barnes, R. J. Use of mean square prediction error analysis and reproducibility measures to study near-infrared calibration equation performance. *J. Near Infrared Spectrosc.* **1999**, *7*, 133−143.

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