

Determination of honey quality components by near infrared reflectance spectroscopy

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Introduction

Honey quality criteria are specified in the International Honey Commission and in the Codex Alimentarius standards (1993).¹ The search for a simple objective method to assess chemical and physical characteristics of honey has received increasing attention during recent years. The lack of simple, reliable and non-destructive methods for the determination of chemical composition in honey has been one of the main obstacles for the development of quality control in both the food industry and commercial trade. Conventional methods involve time consuming, laborious and costly procedures, and chemical analysis methods, relating spectra of analysed samples to specific chemical constituents have been developed.² One such promising technique for honey is Near Infrared Reflectance Spectroscopy (NIRS), a simple and non-destructive technique for analysing quality components such as moisture, sugars and proteins in food and agricultural products.³ The NIR region of the electromagnetic spectrum between the visible and infrared region (750 – 2500 nm), can be used to acquire quantitative information concerning the relative proportions of C-H; N-H and O-H bonds. These are the primary constituents of the organic molecules of which constitute the organic matrix of any food product including honey.⁴ Calibration is the mathematical process required to relate NIR optical measurements to the desired constituent or property used to define the quality of a sample. Accurate calibration is the key to the successful use of the NIR technique and there are a number of major training steps which are essential to obtain a satisfactory working calibration, i.e. sample selection, acquisition of spectra and reference data, pre-treatment of spectral data, derivation of the regression model and validation of the model.³ Rapid and accurate quantitative methods are necessary for quality control and chemical characterisation of honey for trading purposes. There are several reports of the application of Fourier transform infrared spectroscopy (FTIR) and NIRS in relation to the analysis of beet sugar adulteration in honey,² honey quality constituents and in the characterisation of honey according to botanical origin.^{5, 6, 7, 8} The objectives of this study was to explore the use of NIRS to analyse the main chemical parameters in honey, such as moisture (M), electric conductivity (EC), pH, colour and hydroxymethylfurfural (HMF).

Materials and Methods

Honey samples (n=110) were collected from different locations across Uruguay to include different botanical origin, soil characteristics and regions. The samples were kept in the dark, at

room temperature 20 – 25 °C, in plastic jars before chemical and NIRS analysis. All the samples were fresh (< 1 months old) and non-crystallised. Chemical analyses were performed following international recommendations.^{1,9} Samples were analysed for moisture (M) using a refractometer (ATAGO, Japan).^{9, 10} Electric conductivity (EC) was determined on diluted samples using a conductimeter (Orion Model 105, Orion Research, USA). Extracts of honey samples were analysed for pH using a glass electrode pH meter (Orion Model 230 A, Orion Research, USA).¹¹ The presence of hydroxymethylfurfural (2 – hydroxymethyl – 5 – furaldehyde) HMF was determined by a spectrophotometric method.¹² Colour grading was determined using the mm Pfund scale.¹ All the analyses were done in triplicate, and the mean values of the triplicate were used to obtain the NIRS calibration equations. Samples were scanned from 400 - 2500 nm in reflectance mode (transflectance) at 2 nm intervals in a monochromator (NIRSystems 6500, Silver Spring, MD, USA). Spectral information and calibration were manipulated using Infracsoft International (ISI) version 3.1 software (ISI, Port Matilda, PA, USA). The samples were placed in a small cup (50-mm diameter) and presented as a 0.2-mm thick film (Part number IH - 0355-2, NIRSystems, Silver Spring, MD, USA). Reflectance data were stored in absorbance units as the log of the reciprocal reflectance ($\log 1/R$) (where R: reflectance), using a ceramic disk as reference. The reference scan was collected and stored to computer memory before each sample was scanned. The spectrum of each sample was the average of 32 successive scans. Calibration and prediction models were developed using modified partial least squares (MPLS) regression with internal full cross validation and scatter correction by Standard – Normal Variate (SNV) and detrend.^{13, 14} The type of cross validation used by the ISI is called full cross validation. One or more samples are removed from the calibration samples and used as a prediction set. Cross validation estimates the prediction error by splitting the calibration samples into groups (four in this study). In this study one group was reserved for validation and the remaining groups were used for calibration. The process was repeated until all groups had been used for validation once. The outlier elimination pass was set to allow the computer program to remove outliers twice before completing the final calibration. The sequence of calibration models from one PLS or PCA factor to a specified maximum number of factors is derived on the remaining calibration samples and evaluated on the prediction set when cross validation is performed. Two mathematical treatments were applied 1,4,4,1 and 2,10, 10, 2. Where the first number is the order of the derivative (i.e. one is first derivative of $\log 1/R$); the second number is the gap in data points over which the derivative is calculated; the third number is the number of data points used in the first smoothing and the fourth number refers to the number of nm over which the second smoothing is applied. Calibration statistics calculated include the standard error of calibration (SEC), the coefficient of determination in calibration (R^2_{CAL}), standard error of cross validation (SECV) and the coefficient of determination in cross validation (R^2_{VAL}).¹³ Principal Components (PC) were computed on the spectra and used to rank them according to their Mahalanobis distance. The CENTER program ranks spectra in a file according to their Mahalanobis distance (H statistics) from the average spectra of the file using PC scores.¹³ A sample presenting an H statistics > 3 standardised unit from the mean spectrum was defined as a global H outlier and was then eliminated from the calibration set.

Results and Discussion

Figure 1 shows the mean spectrum and standard deviation for honey samples in the visible and near infrared region. Absorption bands in the near infrared region were related at 818 nm with NH stretch third overtone, at 1204 nm with CH stretch second overtone, at 1468 nm with OH stretch second overtone (water), and at 1940 nm with OH stretch first overtone (water). This absorption band at 2102 nm was assigned to CH deformation and combination or C-O stretch combination

overtones, and at 2276 nm with either CH combination, C-C stretch tones or C-O stretch-combination overtones.¹⁵ The best calibration equations were selected based on the lowest SECV. Table 1 shows the NIRS calibration statistics for the chemical parameters analysed. For the 1,4,4,1 mathematical treatment (M), (EC), (A) and colour (C) had the best calibration statistics yielding an R^2_{CAL} of 0.96 (SECV: 31) g kg⁻¹, 0.94 (SECV: 0.07) mS/cm, 0.95 (SECV: 0.05) and 0.98 (SECV: 4.7) mm Pfund, respectively. An intermediate calibration was obtained for pH with a R^2_{CAL} of 0.88 (SECV: 0.21). By applying the second derivative treatment (2,10,10,2) a lower R^2_{CAL} and a higher SECV were obtained (Table 1). The NIRS statistics for the second derivative treatment were 0.96 (SECV: 32) g kg⁻¹, 0.83 (SECV: 0.13) mS/cm, 0.98 (SECV: 6.9) mm Pfund and 0.87 (SECV: 0.21) for M, EC, A, C and pH respectively. The SECV / SD relationship for the NIRS calibration statistics for the properties was used to evaluate the prediction ability of the calibration models worked for the chemical data. We attempted to classify the suitability of reference methods for NIRS calibration, according to the relationship between the error in analysis and the spread in composition.¹⁶ If a product has a narrow range in composition, or if the error in prediction is large compared with the spread (as SD) in composition, then PLS models finds increasing difficulty in finding stable NIR calibrations. Where the standard error exceeds one-third of the SD of the population, regression can often be misleading. A decreasing order of calibration performance was observed for C (SECV/SD: 0.17); M (SECV/SD: 0.24); EC (SECV/SD: 0.35); pH (SECV/SD: 0.54) and HMF (SECV/SD: 0.62), respectively. Water absorption were the most distinctive feature in NIR region. By contrast, the visible region did not show distinct absorption features, thought to be due the freshness of the sample used. In both mathematical treatments applied, HMF had the poorest NIRS calibration statistics (R^2_{CAL} of 0.67 [SECV: 3.9] and 0.51 [SECV: 4.7] mg kg⁻¹ respectively). HMF formation results from the acid catalysed dehydration of hexose sugars, with fructose being particularly susceptible to this reaction. HMF is considered as a trace component in fresh honey samples and according to the literature, small amounts of HMF (0.6 – 20.0 mg kg⁻¹) are present in fresh honey samples (0.75 – 27 mg kg⁻¹ in the present study). Both the storage conditions and heat treatment increased the HMF values.⁷ In the present study, samples had been collected directly from the farm and immediately analysed, so that the freshness of the samples could explain the lower calibration statistics and the poorer prediction accuracy obtained for HMF. Some authors have found better NIRS calibrations for HMF by analysing commercial honeys containing high levels of HMF (range: 8.2 to 40 mg kg⁻¹).⁷ Electric conductivity utilised as a criterion of the botanical origin of honey and depends on the ash content in fresh honey samples. The magnitude of the calibration and cross – validation errors in these analysis were the results of errors and variability scattering and sampling and chemical sub-sampling analysis, while error in the calibration equation models for HMF was attributed to laboratory error associated with the standard analytical method. With regard to pH, some samples appeared as outliers. Most of the samples with high pH (five samples with pH > 4) were poorly predicted by NIRS. For Honey Standard the maximum moisture content limit is the only compositional criteria required to be fulfilled in the world honey trade.¹ The maximum value suggested for moisture in the Codex Alimentarius is about 210 g kg⁻¹. Honey samples exceeding this value are more likely to ferment. NIRS prediction for M content in fresh honey had an excellent correspondence with the reference method.

Conclusions

Predictions obtained in the present study for colour, A and EC showed the potential of NIRS to predict the concentration of various important constituents or functional properties at lower cost than traditional techniques. Based on these results, NIR reflectance spectroscopy may provide reliable prediction of M, colour, and EC in fresh honey samples with implications for the estimation

of chemical composition and quality control by the beekeepers and trade associations. Further studies will continue to incorporate other properties including botanical origin, contamination and adulteration.

Table 1. Near infrared calibration statistics for chemical parameters in fresh honey.

	n	R ² _{cal}	SEC	R ² _{val}	SECV	T
<i>1,4,4,1</i>						
M	101	0.96	2.7	0.94	3.1	5
EC	105	0.94	0.07	0.88	0.01	9
pH	105	0.88	0.13	0.70	0.21	12
HMF	102	0.67	3.5	0.61	3.9	2
A	106	0.95	0.04	0.90	0.05	10
Colour	110	0.98	3.8	0.97	4.7	5
<i>2,10,10,2</i>						
M	106	0.96	2.5	0.94	3.2	8
EC	104	0.83	0.12	0.80	0.13	2
pH	106	0.87	0.14	0.70	0.21	13
A	92	0.83	0.07	0.80	0.08	2
HMF	101	0.51	4.3	0.43	4.7	2
Colour	102	0.98	4.1	0.93	6.9	9

R²_{CAL}. Coefficient of determination in calibration; SEC: standard error in calibration; R²_{val}: coefficient of determination in cross validation; T: number of PLS terms used to perform the calibration; M: moisture (g kg⁻¹); EC: electric conductivity (mS/cm); HMF: hydroxymethylfurfural (mg kg⁻¹); Colour: mm Pfund, n: number of sample in calibration.

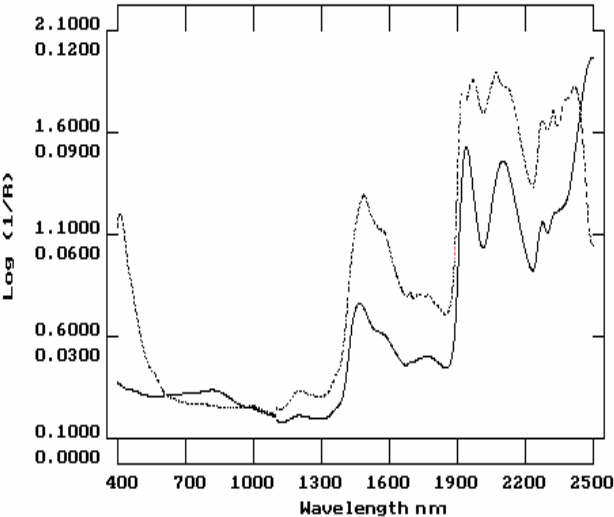


Figure 1. VIS and NIR mean spectrum and standard deviation (dotted line) of honey samples.

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