

CHAPTER 3

Quantitative Determination of Physical and Chemical Measurands in Honey by Near-Infrared Spectrometry*

ABSTRACT

Fourier transform near-infrared spectroscopy (FT-NIR) was evaluated to quantitatively determine 24 different measurands in honey. The reference values of 421 honey samples of different botanical origins were determined by classical physical and chemical methods. Partial least squares regression was used to develop the calibration models for the measurands studied. These calibrations were then validated using independent samples and proved satisfying accuracies for the determination of water (standard error of prediction: 0.3 g/100 g), glucose (1.3 g/100 g), fructose (1.6 g/100 g), sucrose (0.4 g/100 g), total monosaccharide content (2.6 g/100 g) as well as fructose/glucose ratio (0.09) and glucose/water ratio (0.12). The prediction accuracy for hydroxymethylfurfural, proline, pH-value, electrical conductivity, free acidity and the minor sugars maltose, turanose, nigerose, erlose, trehalose, isomaltose, kojibiose, melezitose, raffinose, gentiobiose, melibiose, maltotriose was poor and unreliable. The results demonstrate that near-infrared spectrometry is a valuable, rapid and non-destructive tool for the quantitative analysis of some measurands related to the main components in honey.

3.1. INTRODUCTION

For the general quality control of honey according to the current standards of the Codex Alimentarius (1) and of the European Union (2), several physical and chemical measurands have to be determined, which mostly include water content, enzyme activities of invertase and α -amylase, hydroxymethylfurfural (HMF), electrical conductivity, and sugar composition. At present a specific analytical method has to be applied for each measurand of interest. Moreover, the methods commonly used to determine the chemical composition and the physical properties of honey are laborious and expensive, thus limiting the number of honey samples analysed. To further improve honey quality control it is necessary to develop rapid, simple and accurate methods for the routine quality assessment of honey.

Due to the increased computing performance in the last decades, infrared spectrometry has become a well-established technique for quantitative analysis of food. Infrared spectroscopy has been applied to different fields of honey analysis. The determination of botanical or geographical origin, quality control and detection of adulteration has been discussed in several papers dealing with infrared spectros-

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copy of honey as it presents a rapid and non-destructive approach (3-6).

Near-infrared (NIR) spectrometry has been successfully applied both in transmission and transmittance mode to the quantitative analysis of honey. Transmittance spectroscopy was found to yield sharper peaks and better resolution than reflectance spectroscopy and the calibration performance was found to be 30 - 70 % better. The shortest optical path length tested (1 mm) was found to produce the least saturated spectra in the region between 1300 and 2500 nm thus yielding the lowest standard errors of crossvalidation (SECV) for all components studied (7).

Accurate predictions were obtained for fructose, glucose, sucrose, maltose, water and ash contents as well as for the fructose/glucose and glucose/water ratios in honey samples from different crops (7-13). Furthermore non-compositional characteristics of honey such as electrical conductivity, colour and polarimetric properties (direct polarisation, polarisation after inversion, specific rotation in dry matter and polarisation due to non-monosaccharides) have also been successfully calibrated (10, 14). However, near infrared spectroscopic techniques have not been considered as adequate for the analysis of minor honey components such as HMF, free and lactone acidity as well as pH-value (7, 10). In a calibration limited to avocado honey it was though possible to quantify low concentrations of perseitol (polyol of D-mannoheptulose) (15).

Some authors claim that the isotope ratio between ^{12}C and ^{13}C , used for the detection of cane sugar adulteration, can be determined by NIR. Unfortunately the calibration was restricted to two types of honey and was not validated with adulterated samples (8, 11).

The aim of the present work was to investigate NIR spectroscopy in transfection mode as a rapid analytical tool for the simultaneous quantitative determination of 24 different measurands, used in quality control of honey, based on a large calibration set with as much natural variability as can be expected in practice.

3.2 MATERIAL AND METHODS

3.2.1 HONEY SAMPLES

A total of 421 honey samples were used to establish the global calibration. 352 honey samples from Switzerland collected from seven different crops between 1997 and 2004, including unifloral, (i.e. *Castanea* sp. (n = 27), *Robinia* sp. (n = 19), *Tilia* spp. (n = 13), *Brassica* spp. (n = 25), *Taraxacum* sl. (n = 20), *Rhododendron* spp. (n = 14)), alpine polyfloral (n = 44) and polyfloral (n = 138) as well as honeydew honeys (n = 52) were analysed. Unifloral honeys from *Robinia* sp. (n = 4), *Tilia* spp. (n = 7), *Taraxacum* s.l. (n = 4) and polyfloral honeys (n = 15) of German provenience were included.

In addition polyfloral honey samples from Argentina (n = 3), Chile (n = 5), China (n = 1), Cuba (n = 2), France (n = 6), Greece (n = 1), Hungary (n = 1), Italy (n = 4), Mexico (n = 13), Slovakia (n = 1), Slovenia (n = 1) and Uruguay (n = 1) were included as well. These samples were used to evaluate the calibrations established with samples from Switzerland and Germany.

In order to be able to measure the water content in bakers honey, the calibration range of water content higher than 19 g/100 g was extended up to 24.6 g/100 g by adding water to 17 different polyfloral honey samples. All samples were stored at 4 °C before analysis. They were liquefied in a heating cabinet at 50 °C for 9 h and then allowed to cool to room temperature before analysis.

3.2.2 REFERENCE METHODS

The reference methods used for the quantitative determination of water, electrical conductivity, HMF, pH-value, proline, free acidity as well as various sugars (i.e. fructose, glucose, sucrose, turanose, nigerose, maltose, kojibiose, trehalose, isomaltose, erlose, melezitose and raffinose) were carried out according to the Harmonised Methods of the European Honey Commission (16). Pollen analysis was carried out according to von der Ohe et al. (17) and the botanical origin of the honey samples was determined according to Persano-Oddo and Piro (18). The range of the reference values of the honey samples analysed is indicated in **Table 1**.

Table 1. Reference data ranges of the honey samples

Measurand	Unit	n*	Mean	Minimum	Maximum
Water	g/100 g	382	16.3	13.4	24.6
Fructose	g/100 g	394	37.8	26.4	49.8
Glucose	g/100 g	392	30.1	18.5	40.0
Sucrose	g/100 g	387	0.5	0.0	6.7
Turanose	g/100 g	391	2.2	0.0	5.5
Nigerose	g/100 g	386	2.1	0.0	5.3
Maltose	g/100 g	392	1.6	0.0	4.9
Kojibiose	g/100 g	242	1.0	0.0	2.1
Trehalose	g/100 g	387	0.6	0.0	4.6
Isomaltose	g/100 g	377	0.7	0.0	3.4
Erlose	g/100 g	392	0.6	0.0	4.1
Melezitose	g/100 g	392	0.6	0.0	5.3
Raffinose	g/100 g	397	0.2	0.0	2.2
Gentiobiose	g/100 g	385	0.1	0.0	1.1
Melibiose	g/100 g	392	0.0	0.0	1.3
Maltotriose	g/100 g	392	0.1	0.0	1.9
Monosaccharides sum	g/100 g	393	67.9	44.9	78.2
Fructose/Glucose ratio		391	1.28	0.90	2.11
Glucose/Water ratio		374	1.90	1.09	2.60
Free acidity	meq/kg	376	17	5	44
HMF	mg/kg	388	10	0	112
Proline	mg/kg	370	476	158	1190
Electrical conductivity	mScm ⁻¹	378	0.61	0.10	1.70
pH-value		376	4.4	3.5	6.1

*n: number of samples in cross-validation

3.2.3 NEAR-INFRARED SPECTROMETRY

NIR spectra were recorded using a Büchi NIRLab N-200 spectrometer operated with the NIRLabWare 3.0 software and equipped with a MSC 100 measuring cell with a rotating sample holder (Büchi Labortechnik AG, Flawil, Switzerland) to level out effects of sample inhomogeneity. The measurements were performed at room temperature without temperature control. About 10 g of liquefied honey was poured into a clean glass petri dish and covered with an aluminium plate so defining a 0.75 mm layer of honey between the bottom of the Petri dish and its surface and acting as reflection material. 64 scans with a resolution of 8 cm^{-1} were recorded in transfection mode for each spectrum in the wavenumber range between $4000\text{-}10000\text{ cm}^{-1}$, **Figure 1** shows a typical FT-NIR spectrum of honey. Three replicates of each sample were averaged to obtain a mean spectrum.

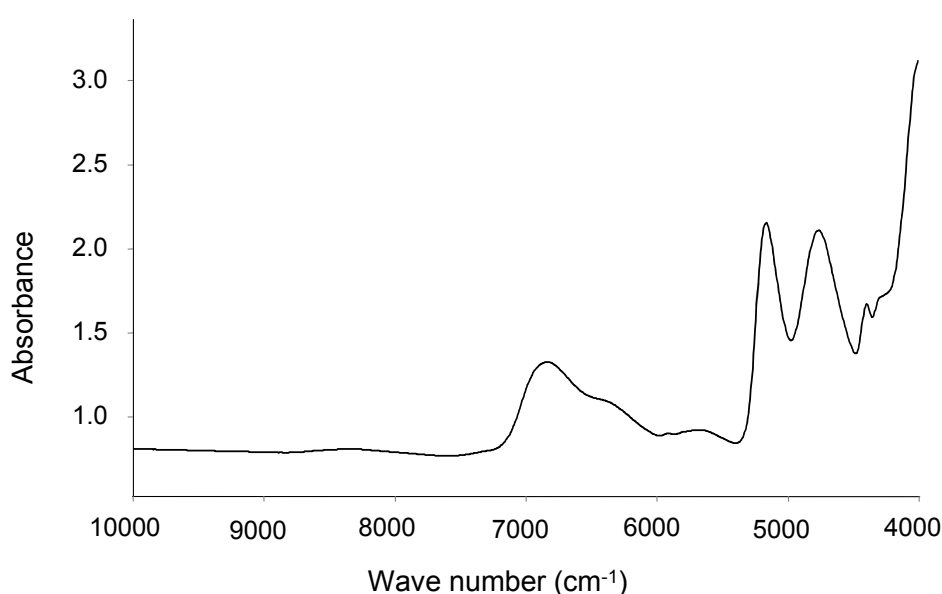


Figure 1. Typical FT-NIR spectrum of a honey sample.

3.2.4 DATA ANALYSIS

The primary interest was to study a 'global' calibration of all honey types considered and to evaluate its performance characteristics with respect to the application in practice where details on the samples are rarely known and are mostly not of interest in quantitative analysis of honey. For the chemometric evaluation, the GRAMS/32 AI Version 6.00 (Galactic Industries Corp., Salem NH, USA) software was used for quantitative analysis by partial least squares (PLS) regression: The calibration models were developed using the PLSplus/IQ add-on in the range between $4200\text{-}10000\text{ cm}^{-1}$ except for water, fructose, turanose, nigerose, kojibiose and isomaltose (see **Table 2**). Information on the interpretation of PLS loading vectors of various measurands can be found in the paper by Qiu et al. (7).

The optimised models were obtained by the "leave one out" cross validation technique based on the minimum predicted residual sum of squares (PRESS). The predictive quality of the models was evaluated by calculating the standard error of cross-validation (SECV) and the standard error of prediction (SEP) in the validation step with independent samples.

3.2.5 CALIBRATION AND VALIDATION

PLS cross-validations were performed to test different calibration models for the prediction of the various measurands. After elimination of spectral and concentration outliers (judged on the basis of Mahalanobis distance > 3) the models were set up with all averaged spectra. For validation (i.e. prediction of samples not included in the calibration) the spectra were split into two data sets. The criterion was to have a statistically sufficient number of validation samples while keeping as many as possible within the calibration set. The samples were arranged according to the numerical value of the measurand under consideration. About every tenth sample was selected for validation. This procedure produced random samples of 30-40 honey samples, which were representative for the distribution of the measurand's values and large enough for statistical validation of the respective PLS-model. The calibration was set up with the remaining spectra not included in the validation set. Validation SEP, coefficients of determination (R^2) between predicted and reference values and prediction bias were calculated (**Table 2**).

3.3 RESULTS AND DISCUSSION

3.3.1 REPEATABILITY LIMITS

The repeatability standard deviations (s) and limits (r_{IR}) of the NIR measurements were calculated based on eleven subsequent analyses of different aliquots of the same polyfloral honey sample (see **Table 2**; repeatability). For comparison the range of repeatability limits (r_{Ref}) from results of interlaboratory studies with the reference methods are listed as far as they are available (**Table 2**) (16).

3.3.2 PREDICTION OF THE MEASURANDS

The resulting standard errors from PLS cross-validation (SECV) and coefficients of determination (R^2) are given in **Table 2**. For the measurands studied, the coefficients of determination in calibration were between 0.009 (maltotriose) and 0.960 (water content) and in validation between 0.078 (HMF) and 0.970 (water content).

3.3.2.1 WATER CONTENT

The water content of honey is the most important measurand for the assessment of ripeness and shelf life, as a honey with a water content higher than 18 g/100 g may be spoiled by fermentation. The NIR method developed allows an accurate determination of this component. The repeatability limit r_{IR} of 0.108 g/100 g is equal to the lowest r_{Ref} of 0.110 g/100 g of the refractometric reference method (16). Moreover, the SEP and the R^2 in validation are with 0.3 g/100 g and 0.970, respectively, the best values of the calibrations performed (**Table 2, Figure 2**). The SEP is in the same range between 0.16 and 0.41 g/100 g as shown by a number of authors (7-13).

Table 2. Cross-validation and validation statistics; repeatability of the method.

Measurand	Unit	Spectral range	Calibration				Validation				Repeatability			
			Number of PLS-factors	^a R ²	^b SECV	^c R ²	Samples in calibration	Samples in validation	^d SEP	R ²	^e Prediction bias	^g s _t	^h r _{IR}	ⁱ r _{Ref}
Water	g/100 g	4200-7200	382	5	0.3	0.960	342	39	0.3	0.970	0.1	0.0	0.1	0.11 - 0.15
Fructose	g/100 g	4200-7200	394	6	1.6	0.759	354	40	1.6	0.810	0.2	0.1	0.4	0.8 - 1.0
Glucose	g/100 g	4200-10000	392	9	1.6	0.814	352	39	1.3	0.884	0.4	0.5	1.4	0.9 - 1.1
Sucrose	g/100 g	4200-10000	387	14	0.6	0.629	290	34	0.4	0.725	0.03	0.1	0.4	0.4
Turanose	g/100 g	4200-7200	391	7	0.7	0.134	350	39	0.6	0.153	0.1	0.0	0.1	0.3 - 0.4
Nigerose	g/100 g	4200-7200	386	13	1.1	0.227	321	38	1.1	0.149	0.3	0.1	0.4	
Maltose	g/100 g	4200-10000	392	10	0.9	0.197	313	37	0.9	0.233	0.2	0.1	0.3	0.5 - 0.6
Kojibiose	g/100 g	4200-7200	242	9	0.3	0.335	207	35	0.3	0.417	-0.03	0.1	0.3	
Trehalose	g/100 g	4200-10000	387	5	0.6	0.426	173	31	0.7	0.463	-0.02	0.0	0.1	
Isomaltose	g/100 g	4200-7200	377	11	0.5	0.313	283	39	0.5	0.420	0.1	0.1	0.1	
Erllose	g/100 g	4200-10000	392	12	0.5	0.462	257	34	0.5	0.664	0.05	0.1	0.2	
Melezitose	g/100 g	4200-10000	392	13	0.7	0.626	223	37	0.8	0.543	0.2	0.3	0.8	
Raffinose	g/100 g	4200-10000	394	11	0.3	0.554	106	31	0.4	0.465	0.04	0.0	0.1	
Gentiobiose	g/100 g	4200-10000	385	8	0.1	0.041	66	36						
Melibiose	g/100 g	4200-10000	392	6	0.1	0.029	79	30						
Maltotriose	g/100 g	4200-10000	392	1	0.2	0.009	58	32						
Monosaccharides sum	g/100 g	4200-10000	393	9	2.5	0.743	353	40	2.6	0.768	0.1	0.5	1.3	
Fructose/Glucose ratio		4200-10000	391	9	0.08	0.833	350	40	0.09	0.820	0.005	0.0	0.1	
Glucose/Water ratio		4200-10000	374	9	0.12	0.814	336	38	0.12	0.849	0.02	0.0	0.1	
Free acidity	meq/kg	4200-10000	376	17	5	0.636	338	38	4	0.737	-0.5	2	4	0.6 - 2.3
Hydroxymethylfurfural	mg/kg	4200-10000	388	15	12	0.435	323	37	13	0.078	0.9	2	5	0.9 - 2.2
Proline	mg/kg	4200-10000	370	18	125	0.588	331	38	125	0.650	7	40	111	6.6 - 24.4
Electrical conductivity	mS cm ⁻¹	4200-10000	378	13	0.17	0.794	339	39	0.14	0.870	-0.003	0.098	0.274	0.002-0.020
pH-value		4200-10000	376	14	0.3	0.622	338	38	0.3	0.657	-0.05	0.1	0.3	0.11 - 0.24

^aPLS-factors: number of partial least squares factors used to build the model in crossvalidation and validation; ^bSECV: standard error of cross-validation, $SECV = \frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n}$ where \hat{y}_i predicted value of spectrum i , y_i reference value of spectrum i and n the number of spectra. ^cR²: coefficient of determination; ^dSEP: standard error of prediction (equation see SECV; but with \hat{y}_i representing a sample not used in calibration); ^ePrediction bias: mean difference between predicted and reference values; ^fRepeatability calculated from 10 predicted values obtained by applying the same calibrations as used for the estimation of the SECV; ^gs_t: repeatability standard deviation in FT-IR spectrometry; ^hr_{IR}: repeatability limit of FT-IR spectrometry; ⁱr_{Ref}: repeatability limit of reference methods from (16)

3.3.2.2 SUGARS

As honey is a complex mixture of various sugars, it is particularly difficult to quantify all sugar types present at low concentrations by infrared spectroscopy. For the R^2 for the main sugars fructose respectively glucose, sufficiently high coefficients of determination of 0.810 and 0.884 and low standard errors both in cross-validation (SECV) and validation (SEP) of 1.6 and 1.3 g/100 g respectively were obtained, indicating that they can be determined by near-infrared spectroscopy with a satisfying accuracy (**Table 2, Figure 2**). The prediction accuracy of fructose and glucose concentrations found in this study is comparable to the findings of previous authors (7, 9, 12, 13, 15).

The sucrose content in honey is defined by maximum limits described in Codex Alimentarius (1) and European Union (2) standards. Moreover it is useful for the determination of the botanical origin (18). The prediction accuracy (SEP: 0.36 g/100 g; R^2 : 0.725) is in the same range as found by Qiu et al. and Ha et al. (7, 12) allowing rough estimation of the sucrose content.

The fructose/glucose ratio and the glucose/water ratio are useful for the identification of the botanical origin of honey (18, 19). The prediction of the former with a SEP of 0.09 and a R^2 of 0.820 was accurate but slightly inferior to the findings of previous studies (SEP: 0.042 to 0.06) (8, 11, 13). However those calibrations were mainly established with acacia honey or based on very few samples. The glucose/water ratio could be predicted with an SEP of 0.12, which is higher than the one found by Pierard et al. (SEP: 0.047) (13) thus allowing only a rough estimation. These two measurands are used for the assessment of crystallisation tendency of honey. honeys with a fructose/glucose ratio higher than 1.3 will crystallize slowly or remain liquid. honeys with a glucose/water ratio of 1.7 or lower will not crystallise. honeys with a ratio between 1.7 and 2.0 will crystallise slowly within one year and honeys with a glucose/water ratio of 2.1 or greater will crystallise fast (20 - 22). However the crystallisation tendency of honey depends also on the amount of seed crystals, heat treatment and storage conditions (22).

The total monosaccharide content (sum of fructose and glucose) is useful for the discrimination of some unifloral honeys and between honeys of nectar and honeydew origin (18, 23, 24). The monosaccharide content could be determined with a satisfying accuracy (SEP: 2.6 g/100 g; R^2 : 0.768). The squared standard error of prediction of the total monosaccharide content corresponds to the squared sum of the SEP of the individual sugars. Our finding corresponds to that found for acacia honey (SEP: 1.760; R^2 : 0.772) by Cho et al. (8) and by mid-infrared spectrometry (SEP: 2.1; R^2 : 0.816) (3).

Minor sugars may contribute to the authentication of some unifloral honeys (25-29) and to the determination of adulteration (30-33). The analysis of the disaccharides maltose, isomaltose, kojibiose, turanose, trehalose and nigerose present in small amounts as well as the trisaccharides erlose and melezitose show a SEP between 0.3-0.8 g/100 g and an R^2 between 0.149-0.664. Concerning gentiobiose, melibiose and maltotriose no calibration at all could be established. This means that near-infrared spectroscopy does not allow an accurate prediction of these minor sugars (**Figure 2**, melezitose). This is caused by the low concentration of these components, by the insufficient separation of these sugars by HPLC and the non-specific absorption bands in NIR. In a calibration with fewer samples a sufficiently accurate prediction of maltose (SEP: 0.28 g/100 g, R^2 : 0.93) was obtained by Qiu et al. (7).

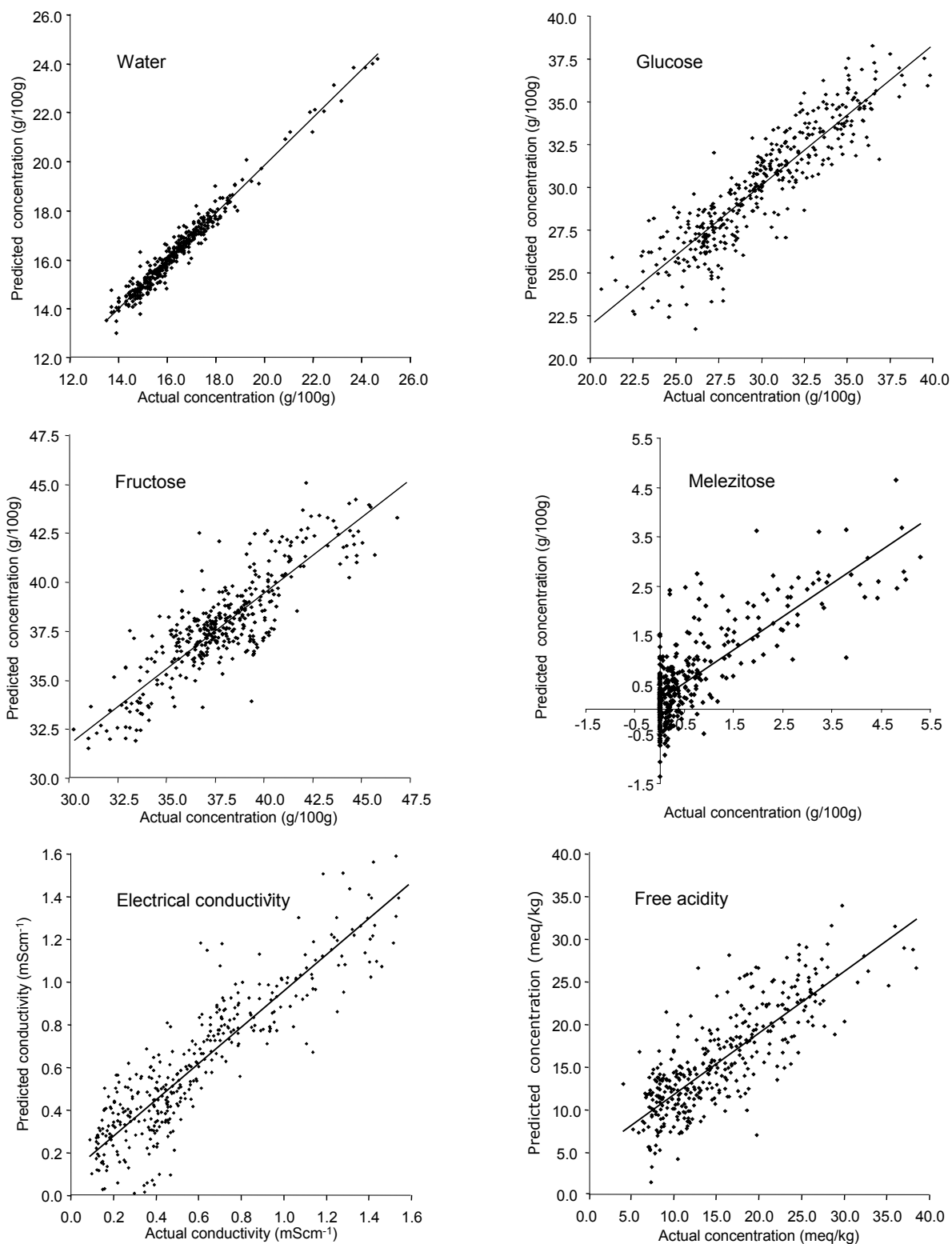


Figure 2. Calibration plots (predicted values from cross-validation)

In the present model the large number of samples considered and their diverse botanical origins are assumed to increase the spectral variability resulting in lower prediction accuracy. It may be improved when individual calibrations would be set up for different types of unifloral honeys. An example may be the good estimation of disaccharides, trisaccharides and perseitol in avocado honey where the calibration was restricted to this type of unifloral honey (15). In the analytical practice, however, this approach is not useful as the type of honey is rarely known or even completely unimportant.

The relatively long optical path of 1.5 mm resulting in very high absorbances, (low signal to noise ratio in the important spectral ranges) may explain the lower prediction accuracies found in the present study (7, 9).

3.3.2.3 FREE ACIDITY

The organic acid content of honey is characterised by its free acidity. This measurement is useful for the evaluation of honey fermentation. A maximum of 50 meq/kg is defined by the current quality standards. Furthermore it is useful for the authentication of unifloral honeys and particularly allows differentiating nectar from honeydew honeys (34, 35). The reference method using equivalence point titration is not very accurate because of lactone hydrolysis induced during titration. Free acidity in honey can be predicted by NIR with a moderate accuracy (SEP: 4 meq/kg; R^2 : 0.737) (Table 2, Figure 2, free acidity). Our results confirm the findings of Qiu et al. (SEP: 4.39, R^2 : 0.49)(7).

3.3.2.4 HYDROXYMETHYLFURFURAL (HMF)

Fresh honey contains only traces of HMF, which is an important criterion for the evaluation of storage time and heat damage. Most of the honey samples analysed were fresh as the median of the HMF content was 5 mg/kg. In order to extend the calibration range to some severely heat damaged samples with a HMF content of up to 112 mg/kg were also analysed. For the calibration range studied the predictive power was found to be very low and unreliable (SEP: 13 mg/kg; R^2 : 0.078). NIR spectroscopy is therefore not adequate for the determination of the HMF content in honey (10). More promising findings (SEP: 1.72 and 3.32 mg/kg) of other authors are restricted to calibrations on the very light coloured acacia honey where the increase of HMF would probably positively correlate with a darkening of the colour during processing (8, 11).

3.3.2.5 PROLINE

The proline content in honey is related to the degree of nectar processing by the bees. It is therefore often used as an indicator of honey adulteration (36). The coefficient of determination is rather low (R^2 : 0.650). The repeatability limit of the proline determination ($r_{IR} = 111$ mg/kg) is considerably higher than the lowest value of the photometric reference method ($r_{Ref} = 6.6$ mg/kg). The determination of proline by NIR is therefore not possible (SEP of 125 mg/kg).

3.3.2.6 ELECTRICAL CONDUCTIVITY AND PH-VALUE

Electrical conductivity and pH-value reflect the mineral content, and the hydronium ion activity of honey. The electrical conductivity is used to distinguish between floral and honeydew honeys according to the current standards (1, 2). Moreover it is also the most important physico-chemical criterion for the authentication of unifloral honeys (37-39).

The pH-value can be used for the discrimination between floral and honeydew honey (35), for the authentication of unifloral honeys (19) and for the differentiation of several honeydew honeys (40).

The non infrared active characteristics of honey such as electrical conductivity and pH-value are not accurate in validation, SEP's being 0.14 mScm^{-1} and 0.3, and R^2 of 0.870 and 0.657, respectively (**Table 2, Figure 2** free acidity and electrical conductivity). These results partly confirm those obtained by Cozzolino and Corbella (electrical conductivity SEP: 0.010 mScm^{-1} , R^2 0.88; pH-value SEP: 0.21, R^2 : 0.70) (10). The repeatability limits of determination by NIR (r_{IR} 0.274 mScm^{-1} and 0.3) are distinctly different of the reference methods that are $0.002\text{--}0.020 \text{ mScm}^{-1}$ respectively $0.11\text{--}0.24$, indicating the basic difficulty of NIR spectrometry applied to the determination of properties not directly related to the gross composition of individual samples even if the correlation between IR absorption and reference values is not lower than for the abundant components. This difficulty arises from the physical principle of the NIR absorption as a very weak interaction between radiation and matter as well as the fact that conductivity and pH-value are properties induced by very small quantities of matter. The (weak) correlations observed between reference values and absorption in some spectral regions are examples of statistical 'nonsense correlations'. Near infrared spectroscopy therefore allows only a rough estimation of the electrical conductivity and pH-value in honey. These two measurands are highly correlated ($r=0.792$; correlation matrix not shown). This is explained by the fact that the various organic acids in honey are at least partially dissociated and therefore act as electrolytes and proton donors.

3.3.3 VALIDATION OF A CALIBRATION ESTABLISHED ON THE BASIS OF SAMPLES FROM SWITZERLAND AND GERMANY WITH SAMPLES FROM OTHER COUNTRIES

A new calibration was set up using all samples except those collected outside Switzerland and Germany. The model was validated with the remaining 37 samples including polyfloral honeys from Argentina, Chile, China, Cuba, France, Greece, Hungary Italy, Mexico, Slovakia, Slovenia and Uruguay. For the measurands studied, all SEP values decreased considerably thus indicating that for maximum accuracy a calibration has to be set up with samples representing all honey types and geographical origins of interest (**Table 3**).

3.4 CONCLUSIONS

The calibration models developed proved satisfying accuracies for the determination of the content of water, glucose, fructose, sucrose, total monosaccharides as well as the fructose/glucose and glucose/water ratios. The prediction accuracies for minor compounds such as HMF and proline, free acidity and the sugars maltose, turanose, nigerose, erlose, trehalose, isomaltose, kojibiose, melezitose, raffinose, gentiobiose, melibiose and maltotriose as well as non infrared active measurands such as pH-value and electrical conductivity, were low and unreliable.

NIR showed for most measurands a better repeatability than mid-infrared spectroscopy (MIR) but only about half the accuracy (3) partially due to less specific absorption bands in the near-infrared region. These differences may also be due to the very high number of samples increasing the variability within the sample set of the NIR calibration (various geographical and botanical origins).

Table 3. Validation statistics of the prediction of measurands of honey samples collected outside Switzerland and Germany based on a calibration established using only Swiss and German samples.

Measurand	Validation with samples from outside Switzerland and Germany					
	Samples in calibration	Samples in Validation	Number of factors	SEP	R ²	Prediction bias
Water	350	37	6	1.1	0.277	0.25
Fructose	357	37	6	1.7	0.716	-0.23
Glucose	356	36	9	1.5	0.838	-0.04
Sucrose	352	37	14	1.1	0.071	1.74
Melezitose	200	37	13	0.8	0.316	0.30
Fructose/Glucose ratio	355	36	9	0.1	0.775	-0.01
Glucose/Water ratio	337	37	9	0.1	0.620	-0.01
Free acidity	339	37	16	7	0.376	13.86
Proline	333	37	17	192	0.349	223
Electrical conductivity	343	36	14	0.29	0.575	-0.04
pH-value	340	37	14	0.4	0.330	-0.62

For more accurate predictions separate calibration models could be set up for different types of unifloral honeys or at least for the main types honeydew and floral honeys. However, the botanical origin of honey is rarely known by the time when quantitative measurements are performed.

As several of the above mentioned measurands can be determined simultaneously with a satisfying accuracy, the technique is useful as a screening tool for the evaluation of the botanical origin of honey in combination with pollen analysis or may even allow a determination of some types of unifloral honeys by spectroscopic means alone (4). At least a reliable differentiation between floral and honeydew honeys can be assumed as an accurate prediction of polarimetric properties can be performed (14).

The determination of measurands such as sucrose and fructose/glucose ratio is valuable for assessing adulteration by sucrose and to predict honey crystallisation tendency. However near-infrared spectrometry does not allow a quantitative determination of HMF and enzyme activities, two criteria particularly important for honey trade, i.e. for the evaluation of storage and heat damage.

The main advantage of NIR combined with multivariate calibration algorithms such as PLS is to simultaneously gain quantitative information on several measurands used for quality control of honey within a short time and a single measurement. Once the calibrations are established NIR spectroscopy allows a rapid analysis of the water, glucose, fructose, sucrose, total monosaccharide contents, fructose/glucose ratio and glucose/water ratio in honey at low cost.

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