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Authentication of the Botanical and Geographical Origin of Honey by Mid-Infrared Spectroscopy

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The potential of Fourier transform mid-infrared spectroscopy (FT-MIR) using an attenuated total reflectance (ATR) cell was evaluated for the authentication of 11 unifloral (acacia, alpine rose, chestnut, dandelion, heather, lime, rape, fir honeydew, metcalfa honeydew, oak honeydew) and polyfloral honey types ($n = 411$ samples) previously classified with traditional methods such as chemical, pollen, and sensory analysis. Chemometric evaluation of the spectra was carried out by applying principal component analysis and linear discriminant analysis, the error rates of the discriminant models being calculated by using Bayes' theorem. The error rates ranged from <0.1% (polyfloral and heather honeys as well as honeydew honeys from metcalfa, oak, and fir) to 8.3% (alpine rose honey) in both jackknife classification and validation, depending on the honey type considered. This study indicates that ATR-MIR spectroscopy is a valuable tool for the authentication of the botanical origin and quality control and may also be useful for the determination of the geographical origin of honey.

KEYWORDS: Honey; unifloral; polyfloral; botanical origin; geographical origin; authenticity; quality control; Fourier transform infrared spectroscopy; FT-IR; ATR; chemometrics

INTRODUCTION

According to the Codex Alimentarius Standard for Honey (*1*) and the European Union Council Directive (*2*) relating to honey, the use of a botanical designation of honey is allowed if it originates predominately from the indicated floral source. It may also be designated by the name of a geographical region if it was produced exclusively within the area referred to (*1*, *2*).

The overwhelming majority of the honeys on the market contain significant nectar or honeydew contributions from several plant species and are therefore called polyfloral or multifloral honeys. Normally, they are just designated with the word "honey". Probably no honey produced by free-flying bees is purely unifloral. The term unifloral honey is used to describe honey in which the major part of the nectar or honeydew is derived from a single plant species. Honey composition, flavor, and color vary considerably depending on the botanical source it originates from (*3*).

The physical, chemical, and pollen analytical characteristics of the most important unifloral honeys have been described in various papers (*3*−*7*). Unlike the unifloral honeys, the polyfloral honeys do not express distinct physical or chemical characteristics but a huge variability regarding all measurands, which makes their authentication particularly difficult.

The interest for the production of unifloral honeys is related to higher consumer preference for some honey types, leading to a commercial interest of the beekeepers. Increasing interest in the therapeutic or technological uses of certain honey varieties may also contribute to the demand for a reliable determination of their botanical origin.

Botanical Origin. Until now, a reliable determination the botanical origin can be achieved only by a global interpretation of sensory, pollen, and physicochemical analyses carried out by experts (*4*, *8*, *9*). However, the uncertainty related to the interpretation of pollen analytical results, originating from a number of different factors, demands the development of new analytical methods (*10*).

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A number of new analytical methods combined with multivariate data analysis have been proposed to determine the botanical origin of honey. They are based on physical and chemical measurands for the quality control of honey (*11*, *12*) sometimes in combination with the determination of mineral content (*13*), as well as carbohydrate composition (*14*), amino acid composition (*15*), mass spectrometry or metal oxide semiconductor based gas sensors (*16*, *17*), differential scanning calorimetry (*18*), pyrolysis mass spectrometry (*19*), and Raman spectroscopy (*20*).

Recently the potential of near-infrared spectroscopy (NIR) to determine the botanical origin of honey was evaluated using a reflectance probe (*21*). Principal component analysis (PCA) and linear discriminant analysis (LDA) were applied for the classification of the honey types studied. Over 80% of acacia, chestnut, and rape honeys were correctly assigned to the corresponding honey type on the basis of the spectroscopic data and Mahalanobis distance in cross-validation, but only a third of the heather honeys considered were correctly classified. However, the number of samples per honey type was very restricted as 13 different unifloral honeys from 9 European countries were studied with a total of 51 samples. No separation into groups according to their geographical origin was found.

Many of the methods mentioned above allow one to clearly discriminate between several types of unifloral honeys (a minority of ≈20%), but none of them accounts for the polyfloral honeys that represent the most important majority (≈80%) of the honeys produced. Thus, the main problem in the authentication of unifloral honeys is to discriminate between polyfloral and unifloral honeys. This means that the above-mentioned methods are inadequate in analytical practice. This also explains why until now none of these methods is commonly applied to the determination of the botanical origin of honey.

Recently Tewari and Irudayaraj claimed that attenuated total reflectance mid-infrared (ATR-MIR) spectroscopy is very promising for the determination of the botanical origin of honey. However, their display of the spectra of different botanical origins is surprising as they differ only in absorption and hardly in shape. On the display of the linear discriminant scores the samples group with an exceptional perfection hardly ever reached by biological samples and could be the result of an overfitting. It would be expected that the so-called "wildflower honeys" (polyfloral honeys) would be much more spread and overlap with the other groups at least in the display of the first discriminant scores. It seems therefore doubtful that the model presented will be valuable in practice (*22*).

Geographical Origin. Pollen analysis is currently used to determine the geographical origin of honey as the pollen in honey reflects the vegetation type from which the nectar has been collected by the bees. In the past many analytical tools such as Raman spectroscopy (*20*), as well as determination of amino acid composition (*23*, *24*), mineral content (*25*, *26*), and sugar or mineral composition sometimes combined with common chemical quality control data (*27*−*29*) together with multivariate data evaluation, have been proposed for the same purpose.

Unfortunately, in most of the above quoted papers the botanical origin of the honey samples has not been determined, or the discrimination between the various geographical origins has not been verified on samples of the same botanical origin. Moreover, the sample sets considered were generally small or limited to a small geographical area. The distinctions found are therefore rather due to differences of the local vegetation type (i.e., to the botanical origin of honey) than to the geographical regions (*30*).

Moreover, criteria related to the main components present in honey are more influenced by the botanical source than by the geographical region. This may explain why no geographical discrimination has been found by near-infrared spectroscopy (*21*). The same fact was also observed in a study using pyrolysis mass spectrometric data in which the variability of the honey types within a country was found to be larger than the variability between the geographical regions of interest (*19*). The presence or absence of certain volatiles analyzed by dynamic headspace GC-MS has been proposed to be specific for some geographical origins as well (*31*). However, the sample set used in this study was very limited and does therefore not allow one to generalize. With relatively small sample sets a discrimination based on mineral or volatile composition between honeys originating from coastal and central provinces of Canada (*32*) and between Hungarian and Italian acacia honeys (*17*) has been shown. These methods have to be validated as analytical tools for the practice.

As several analytical methods have to be used together for a reliable authentication of the botanical origin, it is consequently very time-consuming and costly. In addition, very specialized expertise is needed for the interpretation of the pollen spectrum used for the determination of the geographical origin of honey. Thus, there is a need for new analytical tools that allow both rapid and reproducible authentication of the botanical and geographical origin of honey (*9*, *33*).

Due to the increased performance of computers in the past decades, infrared spectrometry (IR) has become a well-established technique for quantitative food analysis. Concerning honey, it has predominately been applied to the quantitative analysis of different measurands (*34*−*36*). In this context the aim of the current work was to study the infrared spectroscopic characteristics of 11 different honey types and to develop a rapid, low-cost, and reliable method for the authentication of unifloral and polyfloral honeys. As minor nectar contributions from plant species other than the unifloral source may contribute to regional characteristics of unifloral honeys, the potential of ATR-MIR spectroscopy for the determination of the geographical origin of honey was studied as well.

MATERIALS AND METHODS

Sampling and Botanical Classification by Reference Methods. A total of 411 honey samples produced between 1998 and 2004 were collected and stored at 4 °C until analysis. They originated predominately from Switzerland (CH), but samples from Germany (D), Italy (I), Spain (E), France (F), and Denmark (DK) were also considered.

To classify these honey samples, the following measurands were determined according to the harmonized methods of the European Honey Commission (*37*): electrical conductivity, sugar composition, fructose/glucose ratio, pH value, free acidity, and proline content. Pollen analysis was carried out according to DIN 10760 (*38*, *39*).

On the basis of the results obtained with these classical methods, the honey samples were assigned to one of the following 11 honey types according to the criteria of Persano and Piro (*3*): acacia (*Robinia pseudoacacia*) (CH, $n = 17$; D, $n = 6$; F, $n = 3$); alpine rose (*Rhododendron* spp.) (CH, $n = 18$; I, $n = 5$); sweet chestnut (*Castanea sativa*) (CH, $n = 23$; I, $n = 5$; F, $n = 3$); rape (*Brassica napus* var. *oleifera*) (CH, $n = 23$); fir honeydew (*Abies* spp. and *Picea* spp.) (CH, $n = 74$; D, $n = 63$); oak honeydew (*Quercus* spp.) (E, $n = 8$); honeydew from *Metcalfa pruinosa* (I, $n = 14$); heather (*Calluna vulgaris*) (D, $n = 19$; DK, $n = 3$); lime (*Tilia* spp.) (CH, $n = 13$; D, $n = 9$; I, $n = 4$); dandelion (*Taraxacum* s.l.) (CH, $n = 19$; D, $n = 6$; I, $n = 1$); and polyfloral honeys (CH, $n = 75$). In the heterogeneous group of the polyfloral honeys nectar or honeydew contributions from all of the above-mentioned plant species were represented.

Table 1. Jackknife Classification and Validation Tables for the Honey Samples Classified by LDA

	jackknife classification rate (%)											
	acacia	alpine rose	heather	chestnut	dandelion	lime	rape	fir honeydew	metcalfa honeydew	oak honeydew	polyfloral	correct
acacia ($n = 25$)	**100**	0	0	0	0	0	0	0	0	0	0	100
alpine rose ($n = 22$)	0	**95**	0	0	0	0	0	0	0	0	**5**	95
heather ($n = 21$)	0	0	**98**	0	0	0	0	0	0	0	2	98
chestnut ($n = 31$)	0	0	0	**100**	0	0	0	0	0	0	0	100
dandelion ($n = 23$)	0	0	0	0	**100**	0	0	0	0	0	0	100
lime ($n = 25$)	0	0	0	0	0	**88**	0	0	0	0	**12**	88
rape ($n = 22$)	0	0	0	0	**7**	0	**89**	0	0	0	**5**	89
fir honeydew ($n = 130$)	0	0	0	0	0	0	0	**95**	0	0	**5**	95
metcalfa honeydew ($n = 13$)	0	0	0	0	0	0	0	**8**	**92**	0	0	92
oak honeydew ($n = 8$)	0	0	0	0	0	0	0	0	0	**100**	0	100
polyfloral ($n = 75$)	2	**6**	0	3	**11**	**9**	**5**	**5**	0	0	**59**	59

	classification rate in validation (%)										
	acacia	alpine rose	heather	chestnut	dandelion	lime	rape	fir honeydew	metcalfa honeydew	polyfloral	correct
acacia ($n = 8$)	**100**	0	0	0	0	0	0	0	0	0	100
alpine rose ($n = 7$)	0	**100**	0	0	0	0	0	0	0	0	100
heather ($n = 7$)	0	0	**100**	0	0	0	0	0	0	0	100
chestnut ($n = 10$)	0	0	0	**100**	0	0	0	0	0	0	100
dandelion ($n = 7$)	0	0	0	0	**71**	**14**	**14**	0	0	0	71
lime ($n = 8$)	0	0	0	0	0	**100**	0	0	0	0	100
rape ($n = 7$)	0	0	0	0	0	0	**100**	0	0	0	100
fir honeydew ($n = 40$)	0	0	0	0	0	0	0	**98**	0	3	98
metcalfa honeydew ($n = 4$)	0	0	0	0	0	0	0	0	**100**	0	100
polyfloral ($n = 25$)	**8**	**12**	0	**8**	**4**	**28**	0	**14**	0	**26**	26

FT-IR-ATR Spectroscopy. Fourier transform MIR spectra were recorded using a Bio-Rad FTS-7 (Bio-Rad, Cambridge, MA) equipped with a MKII Golden Gate single-reflection ATR accessory (Specac Inc., Woodstock, GA). The measuring cell consisted of a diamond of 2.8 mm in diameter with a refractive index of 2.4 at 1000 cm^{-1}. The depth of penetration of the infrared radiation was 2.0 μm at 1000 cm^{-1} for a sample with a refractive index of 1.5 (approximately the refractive index of honey). The spectrometer was equipped with a deuterated triglycine sulfate (DTGS) detector and was operated at 4 cm^{-1} spectral resolution.

The honey samples were liquefied in a water bath at 55 °C for 8 h and then allowed to cool to room temperature before analysis. After a drop of the sample had been applied on the surface of the diamond, it was left to thermally equilibrate for 4 min. The number of scans per spectrum was selected on the basis of optimal signal-to-noise ratio and acquisition time required. One hundred scans were recorded for each spectrum in the wavenumber range between 4000 and 550 cm^{-1}. Single-beam spectra of all samples were recorded and ratioed against the background spectrum of the clean diamond surface (laboratory air) in order to present the spectra in absorbance. Two spectra were recorded at room temperature using different aliquots of each sample. After each measurement, the diamond was thoroughly washed with demineralized water and dried with a soft tissue. The repeatability was determined by 10-fold measurement of a honeydew honey sample.

Processing of Spectra and Multivariate Analysis. To exclude noisy parts of the spectra only the range between 3718 and 631 cm^{-1} was used for multivariate analysis. After elimination of spectral outliers, PCA was applied to eliminate the spectral collinearity and to reduce the number of variables to 20 PCs (PCA with GRAMS/32 AI, PLSplus/IQ Add-on, Vs. 5.09, Galactic Industries Corp., Salem, NH).

In LDA, the 20 initial PCs were further reduced by backward elimination of principal components on the basis of their partial F values in the discriminant models (SYSTAT version 11, Systat Software Inc., Richmond, VA). To include the variability of single measurements in the model, both spectra of each sample were used in PCA and LDA. The validation was carried out with spectra of one-third of the samples, selected randomly, and not present in the group of samples used to build the model.

The results in jackknife classification ("leave one out" procedure) and validation (**Table 1**) revealed that polyfloral honeys were very often classified into the groups of the unifloral honeys, whereas, inversely, the latter were rarely misclassified into the polyfloral honeys. This observation led to the idea to develop a two-step procedure. In the first step the sample was attributed to one of the 11 honey types considered using an overall discriminant model with as many groups as honey varieties. In the second step this classification was verified by applying several models consisting of a group formed by samples of a given unifloral honey versus a group called "non-unifloral" consisting of all the other samples. Each two-group model was separately built using LDA backward elimination and forward selection. For the verification of the classification by the first model at least the two-group model of the corresponding honey type was used. In addition, one to four two-group models were tested when a misclassification rate of >3% was calculated in jackknife classification or validation tables of the overall model (fields in boldface in **Table 1**). The probabilities for misclassification based on the spectra were calculated by applying Bayes' theorem on the conditional probabilities of disjoint events. The error probabilities cannot be directly taken from **Table 2**; they only quantify the conditional probabilities of correct classification *given* the corresponding honey type. By Bayes' theorem the *posterior*

Table 2. Jackknife and Validation Table for the Honey Samples Classified by the Two-Group Discriminant Models

	jackknife classification				validation unifloral	
	unifloral		non-unifloral			
	n	classification (%)	n	classification (%)	n	classification (%)
acacia	25	100	370	98	8	100
alpine rose	22	91	373	87	7	64
heather	21	98	374	100	7	100
chestnut	31	100	364	99	10	100
lime	25	88	370	80	8	100
dandelion	23	100	372	91	7	100
rape	22	95	373	90	7	100
fir honeydew	130	95	265	98	40	93
metcalfa honeydew	13	92	382	100	4	100
oak honeydew	8	100	387	100		
polyfloral	75	69	320	82	25	26

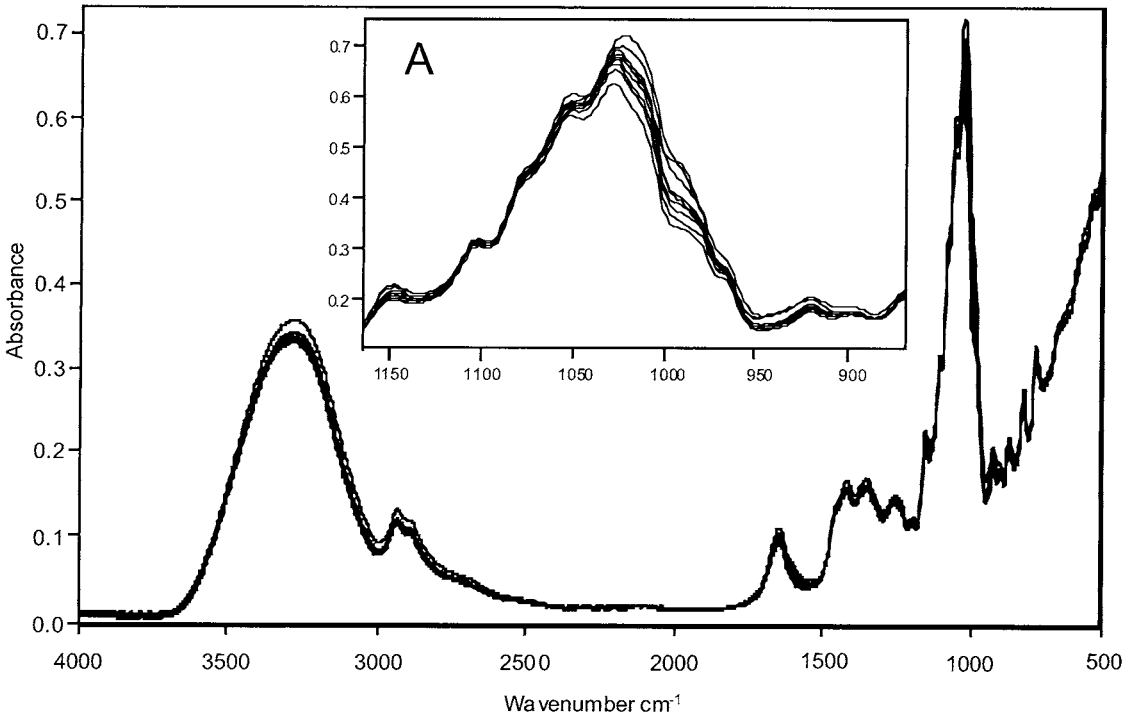

Figure 1. FT-ATR-MIR spectra of different honey types: (**A**) enlargement of the region between 900 and 1150 cm^{-1}.

probabilities of finding the correct honey type given a distinct classification by the discriminant model were calculated, and the error rate is simply the complement to 1.

Geographical Origin. The applicability of FT-IR-ATR spectroscopy for the determination of the geographical origin of honey was evaluated for the honey types when samples from different countries were available. The differences resulting from the botanical origin were studied within the groups of unifloral honeys and between several honey types from Germany and Switzerland by using MANOVA and LDA (SYSTAT version 11, Systat Software Inc.).

RESULTS AND DISCUSSION

Repeatability Limits. The repeatability limit (r_{IR}) of the FT-IR-ATR measurements were calculated at the maximum absorbance at 1024 cm^{-1} from 10 subsequently recorded spectra of different aliquots of the same honeydew honey sample. The average of the maximum intensity of 0.714, the standard deviation of 0.002, a coefficient of variation of 0.3%, and a r_{IR} of 0.006 were found, indicating an excellent repeatability of the method.

FT-IR-ATR Spectra of Different Honey Types. The mid-infrared spectra of the 10 unifloral honey types studied are shown in **Figure 1**. Each spectrum is typical for a given honey type. The most characteristic differences were observed between 800 and 1500 cm^{-1}. The largest variations in the spectra of the honey types were found in the C−O and C−C stretching regions of the saccharides between 950 and 1050 cm^{-1} (**Figure 1A**). Indeed, differences between the saccharide compositions of unifloral honeys have been reported (*3*, *11*, *40*). A more detailed discussion of the vibrational modes of the functional groups in honey can be found elsewhere (*22*).

Botanical Origin. Most of the unifloral honeys revealed very high rates of correct classification of >90% when classified using LDA on PCs of the infrared spectra (**Table 1**). The rates were similar in jackknife classification and validation, demonstrating that the models used were robust. Among the unifloral honeys the lime honeys showed the lowest jackknife classification rate (88%). Twelve percent of the lime honey samples were classified as polyfloral honeys. This may be explained by the variable chemical composition of this honey type as it often contains different amounts of honeydew and thus exhibits variable physical and chemical characteristics. This makes it similar to polyfloral honey that may also contain nectar and honeydew contributions. Rape honey samples were partly classified as dandelion and polyfloral honeys and exhibited the second lowest classification rate (89%). The misclassifications can be explained by the fact that dandelion and rape nectar contribute significantly to polyfloral honeys produced in Switzerland. In validation dandelion honey samples were misclassified to lime and rape honeys. However, the relatively low number of samples does not allow a concluding evaluation. The different honeydew honeys were mostly assigned to the correct group except a few samples of metcalfa honeydew honeys that were misclassified as fir honeydew honeys. However, the number of oak honeydew samples was very small, therefore not allowing a validation.

Even though the samples originated from different geographical origins, they were nevertheless correctly classified according to their botanical origin. Irrespective of their geographical origin the infrared spectroscopic characteristics of honey from various botanical origins seem to be uniform, as samples collected from outside Switzerland grouped among those from Switzerland (**Figure 2**; for better legibility the discriminant scores of only five different honey types are displayed).

It has been clearly shown that it is possible to discriminate between different types of unifloral honeys by infrared spectra and using a single mathematical model. However, this does not mean that the method will be useful in practice as polyfloral honeys are only correctly classified to 59% and are very often misclassified into several types of unifloral honeys. Therefore, the approach using two steps as described under Materials and Methods was tested. After the classification by the general model, one to five two-group models (indicated by boldface type in **Table 1**) were used. The classification rates for the unifloral honeys in the two-group models were generally >90%, whereas the classification rate for the polyfloral honeys ranged

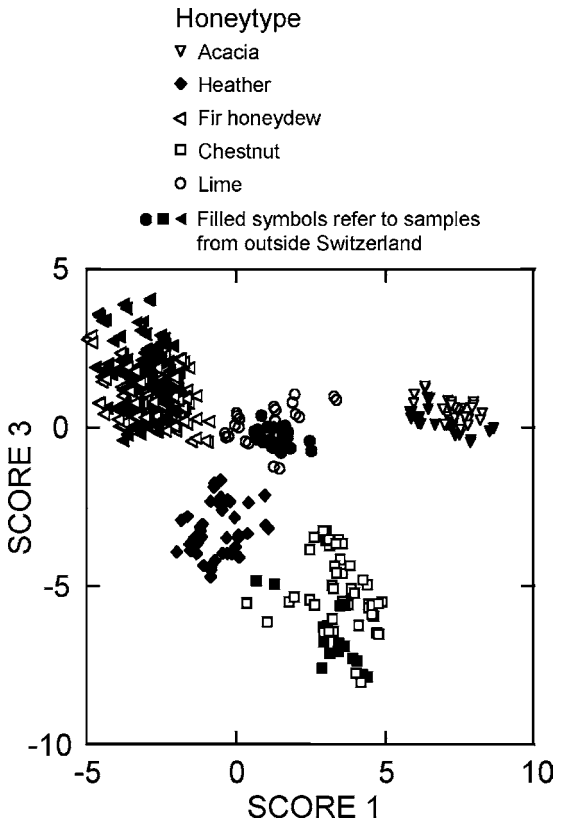

Figure 2. Scatterplot of canonical discriminant scores of different unifloral honeys from LDA (for better legibility, the scores of only five honey types are displayed; all heather honeys originated form outside Switzerland).

between 26 and 82% (**Table 2**). However, as far as the polyfloral honeys are concerned, this is not very important, as we are principally interested in figuring out the unifloral honeys. The high rates of correct classification for both the unifloral and nonunifloral groups considered by the two-group models indicate that the botanical origin can be reliably determined by this procedure.

If the sample is assigned to the same honey type by the overall and the corresponding two-group model, it is very likely that it belongs to this type of honey. If the classifications of the two models do not agree, the sample has to be considered to be of polyfloral origin. When the sample is assigned to the same honey type by both models, the overall model and the corresponding two-group model, and is moreover considered to belong to the nonunifloral groups in all of the other two-group models tested, the honey sample belongs almost certainly to the honey type indicated by the overall model. The respective error rates of this two-step procedure were calculated by using Bayes' theorem. The error probabilities (misclassification of a sample of unknown botanical origin) for the 11 honey types studied except for alpine rose honey were found to be $\leq$3% (**Table 3**). The approach using two successive models allowed a reliable determination of both the polyfloral and unifloral honeys. The classification based on ATR-MIR spectroscopic data and the mathematical models developed are in agreement with the classification using the traditional physical, chemical, and pollen analytical criteria (*3*).

Geographical Origin. Differences in geographical origin were first studied by MANOVA within the groups of samples of the same botanical origin when such samples were available from at least two countries. A highly significant difference was thus found between the geographical origins of all the honey types considered (**Table 4**). When the geographical origins were

Table 3. Error Probabilities for the Classification of Unifloral and Polyfloral Honeys Calculated by Bayes' Theorem

	error probability	
honey type	jackknife	validation
acacia	0.027	0.031
alpinerose	0.083	0.074
heather	$<10^{-3}$	$<10^{-3}$
chestnut	0.016	0.027
lime	0.027	0.019
dandelion	0.015	0.009
rape	0.015	0.009
fir honeydew	$<10^{-3}$	$<10^{-3}$
metcalfa honeydew	$<10^{-3}$	$<10^{-3}$
oak honeydew	$<10^{-3}$	
polyfloral	$<10^{-3}$	$<10^{-3}$

Table 4. Results of MANOVA for the Geographical Origin of the Different Unifloral Honeys

honey type	Wilks' λ	p
acacia	0.002	$<10^{-3}$
alpinerose	0.073	$<10^{-3}$
heather	0.041	0.029
fir honeydew	0.251	$<10^{-3}$
chestnut	0.016	$<10^{-3}$
lime	0.002	$<10^{-3}$
dandelion	0.330	0.014

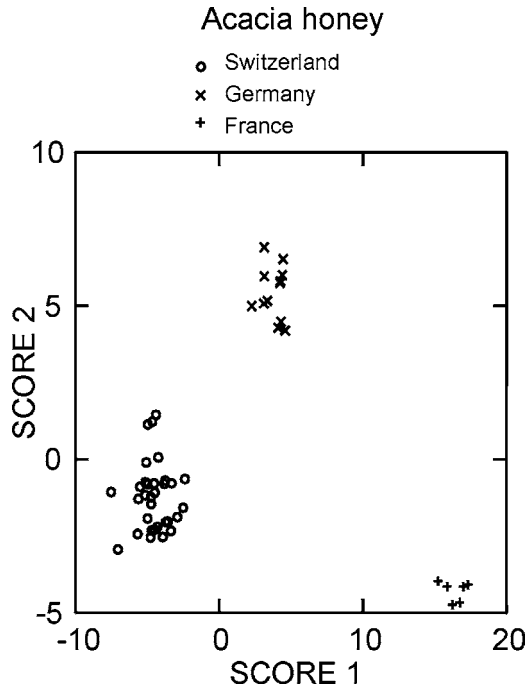

Figure 3. Scatterplot of canonical discriminant scores of acacia honeys of different geographical origins.

modeled by LDA, the spectra were correctly classified at high rates according to their geographical origin: alpine rose, 95%; heather, 77%; chestnut, 98%; lime, 100%; and dandelion, 76%. The spectra of acacia honey samples originating from Switzerland, Germany, and France were all correctly classified and formed groups according to their geographical origin (**Figure 3**). However, the number of samples available from countries outside Switzerland was very limited. Therefore, the effects observed should be verified with a larger set of samples.

Interestingly a difference between fir honeydew honeys of German and Swiss origin could be observed in a larger set of

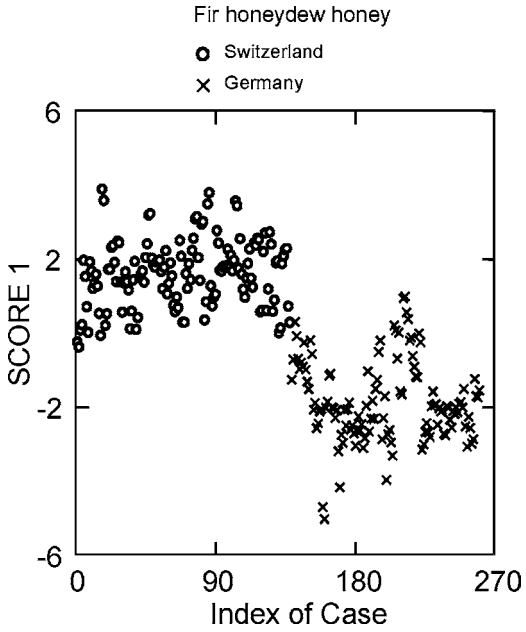

Figure 4. Scatterplot of the canonical discriminant score of fir honeydew honeys of from Germany and Switzerland.

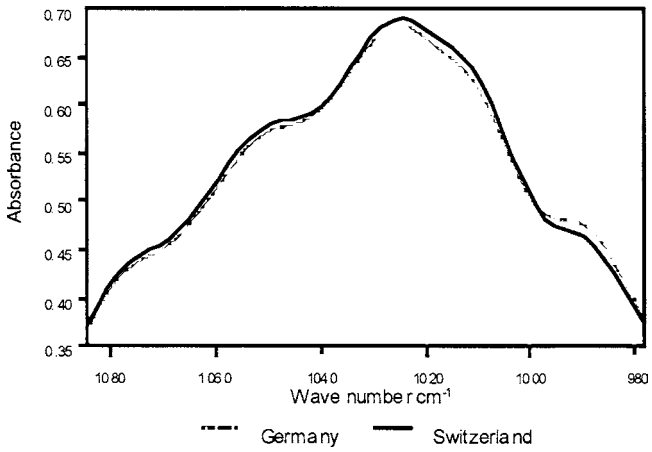

Figure 5. Enlargement of FT-ATR-MIR average spectra of fir honeydew honeys from Germany and Switzerland.

samples originating from several crops. The average jackknife classification rate was 92%. In the plot of the first discriminant scores the Swiss samples generally had positive values and the German samples negative values (**Figure 4**). The overlapping was small considering that all samples originated from an area of only ≈300 km in diameter. In the average spectra of the German and Swiss honeydew honey samples differences were observed especially at the shoulder at 994 cm^{-1} of the distinct band with the maxium at 1024 cm^{-1} resulting from C−O and C−C stretching of the saccharides (**Figure 5**). The average spectra of the German honeydew honeys crossed the average spectra of the Swiss honeydew honeys at 1000 cm^{-1} and showed a more pronounced shoulder at 994 cm^{-1}. These subtle distinctions could be verified by multivariate analysis of the concentration of the various saccharides in honey but probably lie within the measurement uncertainty of the reference method.

To verify whether the geographical origin can also be determined when samples of different botanical origins are considered, LDA was carried out on samples of acacia, lime, dandelion, and honeydew honeys from spruce and fir of both German and Swiss origins. The average rate of correct classification remained quite high at 85% (**Table 5**). When average

Table 5. Percentage of Correct Classification According to Geographical Origin

	jackknife classification matrix		
	Switzerland	Germany	correct (%)
Switzerland	197	32	86
Germany	27	136	83

spectra of the unifloral honeys were compared, all except lime honey showed similar differences as observed between the honeydew honeys from Switzerland and Germany (**Figure 5**).

When LDA was performed on the same dataset using the botanical origin as grouping variable, all spectra were correctly assigned to the corresponding group of unifloral honey, thus indicating that the botanical origin is more significant than the geographical origin. In other words, differences observed and interpreted as resulting from geographical origin may be indirect effects of the botanical origin. In uniforal honeys these differences could originate from small nectar contributions of the accompanying flora that may change with the geographical region where the honey is harvested.

Although absolutely pure unifloral honeys do not exist, the definition of unifloral honey is in fact based on the points of view and descriptions of different analysts. Obviously a certain consensus has been found using the physical, chemical, and pollen analytical criteria for unifloral honeys (*3*−*5*).

The characteristic physical and chemical differences between unifloral and polyfloral honeys are small, and only a few compounds are specific to a given type of honey; the chemometric approach based on a spectroscopic "fingerprint" seems to be more promising than the use of certain marker compounds. The present study shows that ATR-MIR spectroscopy combined with chemometrics offers a valuable approach to the authentication of the botanical origin of honey. The problems related to the determination of the polyfloral honeys can be overcome by the successive use of at least two discriminant models. Whereas previous studies were able to discriminate only between different unifloral honeys, this work demonstrates that unifloral honeys can be authenticated and distinguished from polyfloral honeys. The technique is nondestructive, rapid, easy to use, and not expensive. It needs neither particular sample preparation nor special qualification of the laboratory personnel. Our results show that the authentication of the botanical origin of honey by ATR-MIR spectroscopy and chemometrics is in agreement with the determination using classical criteria. In addition, the same spectra can be used to obtain quantitative information on several measurands used for the routine quality control of honey (*41*).

The present work clearly shows that infrared spectroscopic characteristics of honey are much more dependent on their botanical origin than on their geographical origin. The differences in geographical origin observed in this study should be verified in future investigations with larger sample sets better representing the honeys produced in different geographical regions and by including polyfloral honeys as well. It would certainly be helpful if the geographical origin could be determined within a unifloral honey type, but in principle a method for the determination of the geographical origin should be applicable and validated for all honey types.

A drawback of the current method is that before the botanical origin can be determined routinely, the proposed spectroscopic method needs a considerable amount of preliminary work, to be carried out by specialists, to build the chemometric models based on samples of known botanical origin. However, these models could likely be transferred from an instrument to another

as already demonstrated for the quantitative analysis of various food constituents (*42*−*44*) and substance identification by spectral databases. This remains to be verified in future studies.

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LITERATURE CITED

(1) Codex Committee on Sugars, Codex standard 12, Revised Codex Standard for Honey. *Stan. Stan. Methods* **2001**, *11*, 1−7.
(2) Council Directive 2001/110/EC of 20 December 2001 relating to honey. *Off. J. Eur. Communities* **2002**, *L10*, 47−52.
(3) Persano Oddo, L.; Piro, R. Main European unifloral honeys: descriptive sheets. *Apidologie* **2004**, *35* (special issue), 38−81.
(4) Persano Oddo, L.; Piazza, M. G.; Sabatini, A. G.; Accorti, M. Characterization of unifloral honeys. *Apidologie* **1995**, *26*, 453−465.
(5) Piazza, M. G.; Persano Oddo, L. Bibliographical review of the main European unifloral honeys. *Apidologie* **2004**, *35* (special issue), 94−111.
(6) Moar, N. T. Pollen analysis of New Zealand honey. *N. Z. J. Agric. Res.* **1985**, *28*, 39−70.
(7) Crane, E.; Walker, P.; Day, R. *Directory of Important World Honey Sources. Nectar Plants*; International Bee Research Association: London, U.K., 1984.
(8) Persano Oddo, L.; Bogdanov, S. Determination of honey botanical origin: problems and issues. *Apidologie* **2004**, *35* (special issue), 2−3.
(9) Bogdanov, S.; Ruoff, K.; Persano Oddo, L. Physico-chemical methods for the characterisation of unifloral honeys: a review. *Apidologie* **2004**, *35* (special issue), 4−17.
(10) Molan, P. C. The limitations of the methods of identifying the floral source of honeys. *Bee World* **1998**, *79*, 59−68.
(11) Devillers, J.; Morlot, M.; Pham-Delegue, M. H.; Dore, J. C. Classification of monofloral honeys based on their quality control data. *Food Chem.* **2004**, *86*, 305−312.
(12) Mateo, R.; Bosch-Reig, F. Classification of Spanish unifloral honeys by discriminant analysis of electrical conductivity, color, water content, sugars, and pH. *J. Agric. Food Chem.* **1998**, *46*, 393−400.
(13) Nalda, M. J. N.; Yague, J. L. B.; Calva, J. C. D.; Gomez, M. T. M. Classifying honeys from the Soria Province of Spain via multivariate analysis. *Anal. Bioanal. Chem.* **2005**, *382*, 311−319.
(14) Terrab, A.; Vega-Perez, J. M.; Diez, M. J.; Heredia, F. J. Characterisation of northwest Moroccan honeys by gas chromatographic−mass spectrometric analysis of their sugar components. *J. Sci. Food Agric.* **2002**, *82*, 179−185.
(15) Cotte, J. F.; Casabianca, H.; Giroud, B.; Albert, M.; Lheritier, J.; Grenier-Loustalot, M. F. Characterization of honey amino acid profiles using high- pressure liquid chromatography to control authenticity. *Anal. Bioanal. Chem.* **2004**, *378*, 1342−1350.
(16) Ampuero, S.; Bogdanov, S.; Bosset, J. O. Classification of unifloral honeys with an MS-based electronic nose using different sampling modes: SHS, SPME, and INDEX. *Eur. Food Res. Technol.* **2004**, *218*, 198−207.
(17) Benedetti, S.; Mannino, S.; Sabatini, A. G.; Marcazzan, G. L. Electronic nose and neural network use for the classification of honey. *Apidologie* **2004**, *35*, 397−402.
(18) Cordella, C.; Faucon, J. P.; Cabrol-Bass, D.; Sbirrazzuoli, N. Application of DSC as a tool for honey floral species characterization and adulteration detection. *J. Therm. Anal. Calorim.* **2003**, *71*, 279−290.
(19) Radovic, B. S.; Goodacre, R.; Anklam, E. Contribution of pyrolysis-mass spectrometry (Py-MS) to authenticity testing of honey. *J. Anal. Pyrolysis* **2001**, *60*, 79−87.
(20) Goodacre, R.; Radovic, B. S.; Anklam, E. Progress toward the rapid nondestructive assessment of the floral origin of European honey using dispersive Raman spectroscopy. *Appl. Spectrosc.* **2002**, *56*, 521−527.
(21) Davies, A. M. C.; Radovic, B.; Fearn, T.; Anklam, E. A preliminary study on the characterisation of honey by near infrared spectroscopy. *J. Near Infrared Spectrosc.* **2002**, *10* (2), 121−135.
(22) Tewari, J.; Irudayaraj, J. M. K. Floral classification of honey using mid-infrared spectroscopy and surface acoustic wave based z-nose sensor. *J. Agric. Food Chem.* **2005**, *53*, 6955−6966.
(23) Gilbert, J.; Shepherd, M. J.; Wallwork, M. A.; Harris, R. G. Determination of the geographical origin of honeys by multivariate analysis of gas chromatographic data on their free amino acid content. *J. Apic. Res.* **1981**, *20*, 125−135.
(24) Davies, A. M. C. Amino acid analysis of honeys from eleven countries. *J. Apic. Res.* **1975**, *14*, 29−39.
(25) Latorre, M. J.; Pena, R.; Garcia, S.; Herrero, C. Authentication of Galician (NW Spain) honeys by multivariate techniques based on metal content data. *Analyst* **2000**, *125*, 307−312.
(26) Latorre, M. J.; Pena, R.; Pita, C.; Botana, A.; Garcia, S.; Herrero, C. Chemometric classification of honeys according to their type. II. Metal content data. *Food Chem.* **1999**, *66*, 263−268.
(27) Gonzales Paramas, A. M.; Gomez Barez, J. A.; Garcia Villanova, R. J.; Rivas Pala, T.; Ardanuy Albajar, R.; Sanchez Sanchez, J. Geographical discrimination of honeys by using mineral composition and common chemical quality parameters. *J. Sci. Food Agric.* **2000**, *80*, 157−165.
(28) Sanz, S.; Perez, C.; Herrera, A.; Sanz, M.; Juan, T. Application of a statistical approach to the classification of honey by geographic origin. *J. Sci. Food Agric.* **1995**, *69*, 135−140.
(29) Gomez Barez, J. A.; Garcia Villanova, R. J.; Elvira Garcia, S.; Rivas Pala, T.; Gonzales Paramas, A. M.; Sanchez Sanchez, J. Geographical discrimination of honeys through the employment of sugar patterns and common chemical quality parameters. *Eur. Food Res. Technol.* **2000**, *210*, 437−444.
(30) Cometto, P. M.; Faye, P. F.; Naranjo, R. D. D.; Rubio, M. A.; Aldao, M. A. J. Comparison of free amino acids profile in honey from three Argentinian regions. *J. Agric. Food Chem.* **2003**, *51*, 5079−5087.
(31) Radovic, B. S.; Careri, M.; Mangia, A.; Musci, M.; Gerboles, M.; Anklam, E. Contribution of dynamic headspace GC-MS analysis of aroma compounds to authenticity testing of honey. *Food Chem.* **2001**, *72*, 511−520.
(32) Feller-Demalsy, M.-J.; Vincent, B.; Beaulieu, F. Teneur en minéraux et origine géographique des miels du Canada. *Apidologie* **1989**, *20*, 77−91.
(33) Bogdanov, S.; Martin, P. Honey authenticity. *Mitt. Geb. Lebensmittelunters. Hyg.* **2002**, *93*, 232−254.
(34) Qiu, P. Y.; Ding, H. B.; Tang, Y. K.; Xu, R. J. Determination of chemical composition of commmercial honey by near-infrared spectroscopy. *J. Agric. Food Chem.* **1999**, *47*, 2760−2765.
(35) Garcia-Alvarez, M.; Huidobro, J. F.; Hermida, M.; Rodriguez-Otero, J. L. Major components of honey analysis by near-infrared transflectance spectroscopy. *J. Agric. Food Chem.* **2000**, *48*, 5154−5158.
(36) Lichtenberg-Kraag, B.; Hedtke, C.; Bienefeld, K. Infrared spectroscopy in routine quality control of honey. *Apidologie* **2002**, *33*, 327−337.
(37) Bogdanov, S.; Martin, P.; Lüllmann, C. Harmonised methods of the European honey commission. *Apidologie* **1997** (special issue), 1−59.
(38) DIN (German Institute for Standardisation). *Analysis of Honey—Determination of the Relative Frequency of Pollen*; 2002.
(39) von der Ohe, W.; Persano Oddo, L.; Piana, L.; Morlot, M.; Martin, P. Harmonised methods of melissopalynological analysis. *Apidologie* **2004**, *35* (special issue), 18−25.

(40) Cordella, C.; Militao, J. S. L. T.; Clément, M. C.; Cabrol-Bass, D. Honey characterization and adulteration detection by pattern recognition applied on HPAEC-PAD profiles. 1. Honey floral species characterization. *J. Agric. Food Chem.* **2003**, *51*, 3234−3242.

(41) Ruoff, K.; Iglesias, M. T.; Luginbühl, W.; Bogdanov, S.; Bosset, J. O.; Amadò, R. Quantitative analysis of physical and chemical measurands in honey by mid-infrared spectrometry. *Eur. Food Res. Technol.* **2006**, *223*, 22−29.

(42) Feudale, R. N.; Woody, N. A.; Tan, H.; Myles, A. J.; Brown, S. D.; Ferré, J. Transfer of multivariate calibration models: a review. *Chemom. Intell. Lab. Syst.* **2002**, *64*, 181−192.

(43) Fearn, T. Standardisation and calbration transfer for near infrared instruments: a review. *J. Near Infrared Spectrosc.* **2001**, *9*, 229−244.

(44) Holland, J. K.; Kemsley, E. K.; Wilson, R. H. Transfer of spectral data between Fourier transform infrared spectrometers for use in discriminant analysis of fruit purees. *J. Sci. Food Agric.* **1997**, *75*, 391−400.

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