Attempted Confirmation of the Provenance of Corsican PDO Honey Using FT-IR Spectroscopy and Multivariate Data Analysis

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This study investigated the potential of Fourier-transform infrared (FT-IR) spectroscopy and chemometric techniques to produce a mathematical model that would confirm or refute the provenance of honeys claiming to be Corsican. Authentic honey samples from two harvest seasons (2004/2005 and 2005/2006) were collected from Ireland ($n = 2$), Italy ($n = 30$), Austria ($n = 40$), Germany ($n = 36$), mainland France ($n = 46$), and Corsica ($n = 219$). Prior to scanning, samples were diluted with distilled water to a standard solids content (70° Brix). Spectra (2500−12500 nm) were recorded at room temperature using a FT-IR spectrometer equipped with a germanium attenuated total reflectance (ATR) accessory. Standard normal variate (SNV) and first- and second-derivative data pretreatments were applied to the recorded spectra, which were processed using factorial discriminant analysis (FDA) and partial least-squares (PLS) regression analysis. Overall correct classification figures of 82% (FDA) and 87% (PLS) were obtained for a separate validation set comprising samples from both harvests.

INTRODUCTION

Humans have been consuming honey for many thousands of years; it has been valued as a product in its own right as well as being used extensively in baking and confectionary products (*1*). As a much sought after and exploited product, honey has specific quality standards, which are set out in the internationally recognized Codex Standard for honey (*2*). In addition to these chemical and physical specifications, there is also, in the minds of consumers, a perceived link between the quality of a honey and its provenance (*3*). Such a link has price implications for honey, and country of origin labeling on retail honey packs is therefore mandatory in many places, including the European Union (*4*), Australia (*5*), and Canada (*6*). A protected designation of origin (PDO) for agricultural products and feedstuffs in the European Union may be applied to honeys that meet certain defined requirements including characteristics or qualities arising from a particular geographic origin (*7*). Corsican honey is an example of a honey that has been granted a PDO designation. Any honey with such a designation (Miel de Corse/Mele di Corsica) must fulfill certain specific criteria; that is, it must have been collected and separated on the island of Corsica and be the product of the Corsican ecotype *Apis mellifera* L. from spontaneous vegetative associations of the area (*8*).

It is clear that an independent testing method is necessary to confirm honey provenance claims to enforce legal requirements and for confirmation of PDO designation. Traditionally, pollen analysis (melissopalynology) has been used for these purposes, and this technique is considered to be the reference method (*9*). Determination of geographic origin by this method is based on the quantities and types of pollen present in a sample being consistent with the flora of a particular region and with any reference data or descriptions in the literature (*10*). Analyses of phenolic compounds and flavonoids have also shown promise for geographic origin determination (*11*). Trace element concentration, measured by atomic absorption and emission spectrophotometry or neutron activation, may be correlated with the trace element signature of a particular environment by multivariate analysis, and such an approach has previously been used for geographic origin confirmation (*12−15*) as has chemometric analysis of NMR spectral data (*16, 17*). Although these methods have been successful to greater or lesser degrees, they are time-consuming and require considerable sample preparation.

An alternative to these methods is the use of Fourier-transform infrared (FT-IR) spectroscopy and chemometrics. FT-IR spectroscopy is a rapid technique requiring little or no sample preparation as a result of recent advances in sample presentation methods (*18*). Discrimination between honeys from different parts of Europe and South America using NIR and FT-IR spectroscopy has previously been reported (*19, 20*), and FT-IR spectroscopy has been used in studies distinguishing honeys on

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the basis of their floral origin (*21*, *22*), which can be related to geographic origin (*22*).

The objective of this study was to create an FT-IR spectral library of authentic Corsican samples collected over two harvests and develop a mathematical model to describe them; this model was then to be applied to honey samples claiming Corsican or non-Corsican provenance to estimate its predictive success, that is, to investigate the possibility of developing a spectral specification for Corsican honey.

MATERIALS AND METHODS

Samples. Authentic honey samples (*n* = 373) were collected over two harvest seasons (2004/2005 and 2005/2006). The 2004/2005 harvest consisted of honeys from Corsica (*n* = 111), Italy (*n* = 15), Ireland (*n* = 2), Austria (*n* = 18), Germany (*n* = 18), and mainland France (*n* = 18), whereas the 2005/2006 harvest comprised samples from Corsica (*n* = 108), Italy (*n* = 15), Austria (*n* = 22), Germany (*n* = 18), and mainland France (*n* = 28).

After collection and prior to analysis, samples were stored in the dark at room temperature (21 ± 5 °C) in screw-capped glass or plastic jars. Spectral collection took place over two 14 day periods, one in 2005 and one in 2006. Prior to spectral collection, samples were incubated in an air-oven at 45 °C overnight to dissolve any crystalline material and subsequently manually stirred to ensure homogeneity. The solids content of each sample was measured using an Abbé model 2WA (Kernco Instruments, El Paso, TX) benchtop refractometer and adjusted to a standard solids content (70 ± 1 °Brix) using distilled water; this step was necessary to minimize spectral complications from naturally occurring variations in sugar concentration and to avoid spurious classification on the basis of variations in solids content between honeys. Samples were removed from the air-oven and left to equilibrate to room temperature for approximately 1 h before spectral measurement.

Instrumentation. Spectra were collected on a Bio-Rad Excalibur series FTS 3000 FT-IR spectrometer (Analytica Ltd., Dublin, Ireland); samples were in random order. Instrument control and spectral collection were performed using WIN-IR Pro (v. 3.0) software. Brix-adjusted samples were applied to an in-compartment benchmark attenuated total reflectance (ATR) trough plate using a 45° germanium crystal with 11 internal reflections (Specac Ltd., Kent, U.K.) so as to obtain a maximum absorption of approximately 0.3 at the highest peak, which occurred at approximately 1042 cm^{-1}/9597 nm; this target value was achieved by varying the coverage of the crystal by the samples using a glass rod. Sixty-four scans were co-added for each sample at a nominal resolution of 4 cm^{-1}. A single-beam spectrum of each sample was collected and ratioed against a background of air. Spectra were truncated to 800–4000 cm^{-1} and then converted to a wavelength scale (2500–12500 nm) using Win-IR Pro software.

Between samples, the ATR crystal surface was cleaned with Triton X-100 solution (1% w/w), rinsed with distilled water, and dried with soft tissue. The spectral baseline recorded by the spectrometer was examined visually to ensure that no residue from the previous sample was retained on the crystal. All spectra were recorded at a controlled temperature (21 ± 5 °C) but without any nitrogen purge of the sample compartment.

Statistical Analysis. Means of the 64 co-added scans for each sample were used for statistical analysis. Spectra were exported from WIN-IR Pro as GRAMS files (Thermo Galactic, Salem, NH) and imported directly into The Unscrambler (v9.7; CAMO A/S, Oslo, Norway). Principal component analysis (PCA) (*23*) was performed using The Unscrambler on the entire sample set for preliminary data set examination. Data pretreatments examined in subsequent operations were standard normal variate (SNV) (*24*) and first and second derivatives using a quadratic Savitzky–Golay (*25*) filter and segment sizes between 5 and 21 points.

In all chemometric operations, separate calibration and validation sample sets were used. Calibration sets contained an equal number of Corsican and non-Corsican samples so as to minimize the likelihood of any class bias in the models developed. Corsican samples were selected at random, and an equal number of non-Corsican samples was randomly chosen from each of the other countries. Validation sets consisted of all samples not included in the calibration sets.
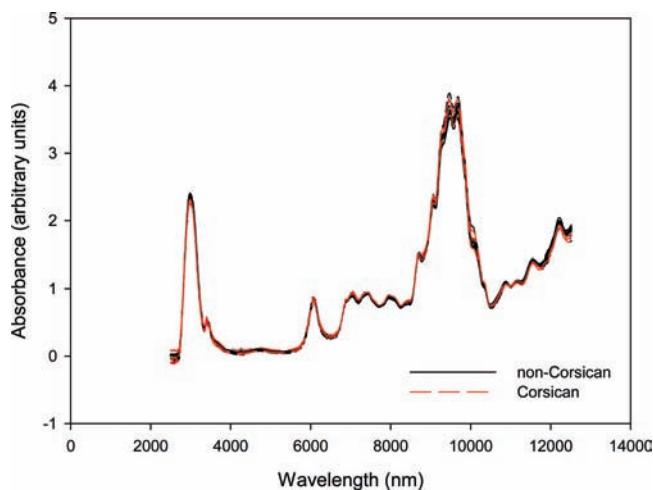
Figure 1. ATR spectra of randomly selected honey samples from different countries and both harvest seasons.

Factorial discriminant analysis (FDA) was executed using the SAI-SIR (*26*) environment for MATLAB. Data files from The Unscrambler were exported as MATLAB files and imported directly into MATLAB (v7.2.3.232 (R2006a), The Mathworks Inc., Cambridge, U.K.). FDA was applied in two steps; first, a PCA was carried out on the spectra, and then FDA was performed on the principal component (PC) scores. The first step creates a set of orthogonal spectral patterns or principal components, and the second step calculates discriminant factors using a stepwise procedure to identify and incorporate those principal components that best discriminate the samples into the relevant groups, in this case country of origin (*27*). The key feature of this technique is that the principal components are incorporated into the discriminant model in such a way as to maximize discriminant ability according to the characteristic of interest rather than in numerical order, PC1 alone, PC1 + PC2, etc. Each sample is assigned to one of the classes of interest; in this study there were two classes, that is, honeys from Corsica and all other honeys.

Partial least-squares discriminant analysis (PLS1-DA), an adaptation of PLS regression that allows it to be used for classification (*28*), optimized by leave-one-out cross-validation was used to discriminate between the honey samples from Corsica and all other regions. Separate dummy variables were generated for each class; a sample was assigned a value of 0 if it was from Corsica and 1 if it was not. PLS models thus developed were used to predict the value of the *Y* variable for each validation sample; given the values of the dummy *Y* variables used, an empirical and not entirely arbitrary value of 0.5 was used as a cutoff for identity confirmation of honeys with predicted *Y* values of < 0.5 deemed to be from Corsica. The outcome of these classifications was expressed as the percentage of correct classifications of each sample class.

RESULTS AND DISCUSSION

As a precursor to chemometric analysis, the spectra of all samples (*n* = 373) were plotted. Visual inspection of the resulting plot (**Figure 1**) did not reveal any usual samples or allow the identification of samples from Corsica.

PCA. PCA was preformed on raw spectral data from both harvests over the full wavelength range recorded (2500–12500 nm) and the resulting scores plot examined. Separation on the basis of harvest season was readily apparent from a plot PC1 and PC3, which accounted for 61 and 10% of the total variance in the spectral data set, respectively. Apart from damage by diseases and pests, the main cause of year-to-year variation in nectar flows from plants is weather; this variation in nectar flow will in turn affect the composition of honey (*29*). Samples from Corsica showed some evidence of forming a cluster in higher order PCs (results not shown), although such clusters were not visibly separate from the other samples in score space.

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A second PCA was performed on spectra over the attenuated wavelength range from 6800 to 11500 nm as this range contains information on sugar composition and was previously reported to be useful in discriminating honey from different origins (*19*). As with the full wavelength range, the scores plot primarily showed clustering on the basis of harvest season; some small degree of separation between Corsican and other samples was suggested in the hyperplane described by PC1 and PC6 (**Figure 2**).

FDA. A number of strategies were employed in the development of factorial discriminant models to this honey data set. In the first instance, FDA was separately applied to samples from each harvest; models thus developed were then used to predict the provenance of honeys in the validation sample set from the same harvest. This approach avoids any interference arising from effects due to harvest season, effects previously demonstrated by PCA on this data set. Results of this approach can be seen in **Table 1**. For the 2004/2005 harvest, correct classification rates for Corsican samples were in the range from 62 to 94%, and correct classification rates for non-Corsican samples were between 61 and 91% depending on the pretreatment and variables used in the
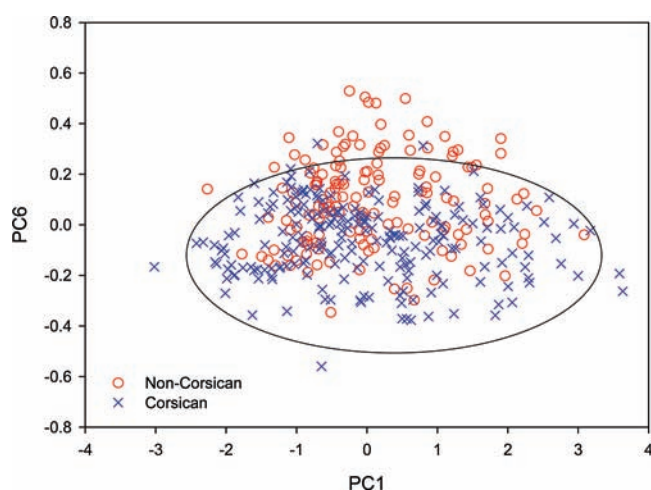
Figure 2. Scores plot of all samples after principal component analysis of raw spectral data in the wavelength range 6800−11500 nm (PC1 versus PC6).

model. In general, using the wavelength range 6800−11500 nm resulted in similar or improved correct classification rates as compared to models developed using the 2500−12500 nm range; average values were increased by at least 4%. Results for the 2005/2006 harvest show that Corsican correct classification rates tended to be lower than the corresponding non-Corsican rates. Corsican samples had correct classifications of 55−77%, whereas 64−93% of non-Corsican samples were correctly classified. Use of the attenuated rather than the full wavelength range did not show any consistent effect on results for this harvest. Average correct classifications for Corsican samples increased and remained the same for non-Corsican samples when the attenuated rather than the full wavelength range was used. Results show that the FDA models developed using samples from a single harvest have correct classification rates of approximately 80% when applied to validation sets from the same harvest.

Single-harvest models from 2004/2005 data were applied to all samples from the second harvest, and results were variable. Depending on the pretreatment used, when correct classification rates for Corsican samples were high, non-Corsican samples had low correct classification rates and vice versa, or else correct classifications for both classes were between 50 and 70%. A similar, varied result was found when 2005/2006 models were applied to 2004/2005 samples. These results imply that for this data set, FDA models from one harvest cannot be used to satisfactorily confirm the provenance of samples from another harvest.

Spectral data from both harvests were then combined and analyzed together; results are shown in **Table 2**. Pretreatments yielding the best results were first derivative with a 21 data point gap and SNV. This was the case for models developed using spectral data in the wavelength ranges 2500−12500 and 6800−11500 nm. First-derivative data pretreatments tended gave better results than second-derivative pretreatments. The highest Corsican and non-Corsican correct classification rates for this combined data set were 79 and 88%, respectively. The rank order of PCs for inclusion in this model (first derivative, 21 point gap, 2500−12500 nm wavelength range) was 5, 8, 9, 11, 4, 10, 16, 17, 13, and 20. It is noteworthy that PC1, PC2, and PC3, which together accounted for 66% of the data set variance, were not selected for this FDA model. This arises in part because the main source of variance in the data arose from harvest year, a feature

Table 1. Factorial Discriminant Analysis Model Performances on Honeys from Individual Harvests

		2004/2005 harvest calibration set: Corsican = 48, non-Corsican = 48 validation set: Corsican = 63, non-Corsican = 23						2005/2006 harvest calibration set: Corsican = 55, non-Corsican = 55 validation set: Corsican = 53, non-Corsican = 28					
		2500−12500 nm % correct classification			6800−11500 nm % correct classification			2500−12500 nm % correct classification			6800−11500 nm % correct classification		
pretreatment	PCs[a]	Corsican	non-Corsican	PCs[a]	Corsican	non-Corsican	PCs[a]	Corsican	non-Corsican	PCs[a]	Corsican	non-Corsican
none	7	79	74	10	78	91	10	75	86	8	77	89
1st derivative												
5 point gap	10	83	83	8	86	87	7	70	86	10	66	86
9 point gap	10	89	74	10	87	87	8	74	86	8	68	86
13 point gap	10	84	83	6	94	83	10	68	89	9	75	86
21 point gap	7	89	83	7	90	83	8	70	89	9	77	82
2nd derivative												
5 point gap	9	62	61	9	81	70	10	55	64	7	58	75
9 point gap	10	83	78	10	90	78	5	58	71	7	66	89
13 point gap	10	84	83	6	90	74	6	64	89	6	70	86
21 point gap	9	90	87	6	83	87	9	64	86	10	74	86
SNV	7	79	70	10	84	91	10	75	93	10	75	79
av		82	78		86	83		67	84		71	84

[a] Number of principal components in the model.

that is not relevant for classification on the basis of geographic origin. The predominance of higher order PCs may be explained on the basis that the differences between honeys from the regions sampled may be expected to be small in terms of both molecular composition and proportionality; such small compositional effects will be extracted only in higher order PCs.

The spectral pattern of the discriminant factor for this best model was examined to try to identify wavelengths that contributed to the two-class discrimination. The first spectral feature of note is a maximum at 3049 nm, which is likely to arise from O−H stretching vibrations. A minimum at 6020 nm and a maximum at 6334 nm may correspond to the H−O−H stretching vibration, which occurs at approximately 6060 nm in a typical FT-IR spectrum of honey (*19*, *30*). Many of the other spectral features present correspond to molecular vibrations associated with saccharides (*22*). A prominent maximum at 8598 nm and a minimum around 8773 nm may be attributed to molecular vibrations arising from C−O and C−C stretching (*31*), whereas

a minimum at 11654 nm may correspond to a C−H bending vibration (*32*). This examination suggests that absorption due to water may have played a role in discrimination of samples despite the dilution of all samples to 70 °Brix; the instrument used was accurate to only ±1 °Brix. The attenuated wavelength range examined does not include the water absorption regions, and classifications based on both wavelength ranges did not differ greatly, so it is unlikely that any small variation of water content had a large impact on the models. The appearance of water as a significant factor may relate to slight changes in its absorbance pattern arising from small differences in content of specific saccharide and other honey components. It should be borne in mind that attribution of these spectral features is difficult by virtue of the chemometric approach used and further complicated by the use of first-derivative spectral data in the model (*33*); any inferences drawn are therefore tentative.

PLS1-DA. Spectra were analyzed by PLS1-DA in a number of ways, similar to the approaches described above for FDA; samples from the first harvest season (2004/2005) were analyzed on their own, as were the samples from the second harvest season (2005/2006). Samples from one harvest were used to predict the identity of samples from the other harvest, and finally the two sets were combined and analyzed together. Two wavelength ranges, the entire recorded range (2500−12500 nm) and an attenuated range (6800 − 11500 nm), were examined.

Table 3 outlines the performance of PLS1-DA models on spectral honey data and on data with first- and second-derivative and SNV pretreatments from the individual harvest seasons. The best performing model for the 2004/2005 harvest correctly classified 90% of the Corsican samples and 91% of the non-Corsican samples. This model was developed using attenuated spectra with a second-derivative pretreatment (13 data point gap, number of PLS loadings = 6), although the results do not vary greatly with wavelength range or pretreatment used. Models for the 2005/2006 harvest do not perform as well as their counterparts from the previous harvest season with correct classifications of between 57 and 79% for Corsican samples and 64 and 89% for non-Corsican samples. Use of the attenuated range improved results for some pretreatments, whereas using the entire spectral range produced better results for other pretreatments. For both harvests, correct classification rates of between 70 and 90% show

Table 2. FDA Model Performances Using Samples from Both Harvests

		calibration set: Corsican = 103, non-Corsican = 103 validation set: Corsican = 116, non-Corsican = 51				
		2500−12500 nm % correct classification			6800−11500 nm % correct classification	
pretreatment	PCs[a]	Corsican	non-Corsican	PCs[a]	Corsican	non-Corsican
none	8	78	80	10	78	86
1st derivative						
5 point gap	10	72	84	7	78	90
9 point gap	7	75	80	4	78	84
13 point gap	8	76	88	9	80	84
21 point gap	10	79	88	10	80	86
2nd derivative						
5 point gap	8	60	63	9	72	80
9 point gap	10	60	65	10	78	78
13 point gap	10	71	80	8	80	80
21 point gap	10	72	86	8	83	78
SNV	10	78	88	10	77	86
av		72	80		78	83

[a] Number of principal components in the model.

Table 3. PLS1-DA Prediction on All Honeys from Individual Harvests

	2004/2005 harvest calibration set: Corsican = 48, non-Corsican = 48 validation set: Corsican = 63, non-Corsican = 23						2005/2006 harvest calibration set: Corsican = 55, non-Corsican = 55 validation set: Corsican = 53, non-Corsican = 28					
	2500−12500 nm % correct classification			6800−11500 nm % correct classification			2500−12500 nm % correct classification			6800−11500 nm % correct classification		
pretreatment	L[a]	Corsican	non-Corsican	L[a]	Corsican	non-Corsican	L[a]	Corsican	non-Corsican	L[a]	Corsican	non-Corsican
none	11	79	78	10	83	87	6	79	89	3	70	79
1st derivative												
5 point gap	7	83	83	8	89	91	5	70	86	7	70	89
9 point gap	9	86	91	7	89	91	5	74	86	6	72	86
13 point gap	7	87	91	8	89	87	5	74	89	5	72	82
21 point gap	6	86	91	3	76	78	3	68	86	5	74	79
2nd derivative												
5 point gap	7	70	61	5	83	74	2	60	64	3	55	82
9 point gap	7	76	78	5	84	83	3	57	75	4	62	82
13 point gap	8	84	87	6	90	91	6	66	89	5	66	82
21 point gap	8	84	87	7	92	83	6	72	89	2	62	71
SNV	11	87	91	11	76	91	4	77	86	2	64	79
av		82	84		85	86		70	84		67	81

[a] Number of PLS loadings in the model.

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Table 4. PLS1-DA Prediction of Corsican Honey Samples from Both Harvests

| | | calibration set: Corsican = 103, non-Corsican = 103
validation set: Corsican = 116, non-Corsican = 51 | | | | |
| | | 2500−12500 nm
% correct classification | | | 6800−11500 nm
% correct classification | |
pretreatment	L^a	Corsican	non-Corsican	L^a	Corsican	non-Corsican
none	8	79	90	10	85	86
1st derivative						
5 data points	6	81	88	6	81	92
9 data points	7	83	92	8	80	92
13 data points	7	84	92	3	68	75
21 data points	11	84	86	9	82	86
2nd derivative						
5 data points	5	68	75	5	72	82
9 data points	7	83	88	5	78	88
13 data points	7	85	92	7	79	88
21 data points	7	79	92	8	83	88
SNV	8	83	92	2	68	78
av		81	89		78	86

a Number of PLS loadings in the model.

that the PLS models have the potential to distinguish between Corsican and non-Corsican samples within the same harvest with an accuracy level that may be commercially useful.

A further test applied to the above models was to use them to predict the provenance of samples from the other harvest. As with FDA, depending on the pretreatment applied, models produced high correct classification rates for Corsican samples with low correct classification rates for non-Corsican samples and vice versa, although the models developed using variables 2500−12500 nm with no pretreatment or SNV pretreatment produced better results.

It is unrealistic to assume that Corsican samples from a particular harvest could represent all past and future samples from Corsica. New discriminant models were developed using the previously employed calibration samples from each harvest. Validation results for these models can be seen in **Table 4**. Corsican samples had correct classifications of 68−85%, whereas non-Corsican samples generally had higher correct classifications ranging from 75 to 92%. The best performing model involved a second-derivative (13 data point gap, PLS loadings = 7) pretreatment and was developed using the wavelength range 2500−12500 nm.

The regression coefficients of this model were examined and, as with the discriminant profile for FDA, definitive spectral attributions cannot be made as a derivative, spectral pretreatment, in this case second, has been used in model development. In addition, spectral interpretation of regression functions is generally of limited value. As before, features associated with water absorption wavelengths were observed; a minimum observed at 3116 nm may arise from O−H stretching, and the two maxima at 6099 and 6286 nm may correspond to H−O−H stretching (*30*). There appears to be an important feature at 8695 nm, and this most likely corresponds to C−O and C−C stretching, which is found at approximately 8696 nm in the mid-infrared spectrum of honey (*31*). It is notable that the most significant wavelengths in the regression coefficient are similar to the most significant wavelengths in the FDA discriminant profile.

The most successful model correctly classified 85% of Corsican samples and 92% of non-Corsican samples, equivalent to an overall correct classification rate of 87% for the validation set. Although this result may not be sufficiently accurate as a definitive test of provenance, it clearly demonstrates that FT-IR

spectroscopy has the potential to be used as a rapid screening technique, at least in this application. Honey samples used in this work have also been analyzed using NIR spectroscopy and PLS1-DA (*34*); results from this study compare favorably.

The analyses of collected spectra by factorial discriminant analysis and partial least-squares discriminant analysis both showed that within one harvest season, approximately 80% of validation samples could be correctly classified into either of the two classes of interest, that is, honey claiming to have a Corsican provenance and honey not claiming to be from Corsica. Models developed using samples from one harvest applied to samples from another harvest were not as successful in distinguishing between the two classes. When samples from the two harvests were combined, FDA correctly identified between 70 and 80% of samples according to their claimed origin. The PLS1-DA models performed slightly better, with between 80 and 90% of samples being correctly identified.

This study shows the FT-IR spectroscopy and chemometric modeling of the resulting spectra have potential for confirming the claimed provenance of authentic honey samples from the PDO region of Corsica. The largest variation in the data was due to the harvest season to which the honey belonged, and results were better when samples from both harvests were included in the calibration and validation models. The intention was not to develop a model that would identify the geographic origin of an unknown honey sample; such a model would require an exhaustive sampling of world honeys over several harvest years and would not be easily realizable even if it was judged worth attempting. Rather, the goal was to compare spectra of honeys claiming to be from Corsica to the model developed and thereby arrive at a decision confirming this provenance or not. It is recognized that Corsican honey may not have a unique spectral signature and that honeys from other countries or localities around the world may be spectrally similar due to similarities in vegetation. However, a successful outcome to the approach studied would be useful to Corsican honey producers as a quality assurance tool and by, for example, retailers as a screening tool to check honey from suppliers with whom they have an established relationship. It should therefore enhance consumer confidence in such a product.

Before commercial deployment of this method, the sample collection would need augmentation with more samples collected over a larger number of harvests; in this way the effects of weather variations would be incorporated. Parallel studies investigating the effect of storage time, temperature, and exposure to light on spectra would also be required for the creation of robust models.

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