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Geographical Classification of Honey Samples by Near-Infrared Spectroscopy: A Feasibility Study

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The potential of near-infrared (NIR) spectroscopy to determine the geographical origin of honey samples was evaluated. In total, 167 unfiltered honey samples (88 Irish, 54 Mexican, and 25 Spanish) and 125 filtered honey samples (25 Irish, 25 Argentinean, 50 Czech, and 25 Hungarian) were collected. Spectra were recorded in transflectance mode. Following preliminary examination by principal component analysis (PCA), modeling methods applied to the spectral data set were partial least-squares (PLS) regression and soft independent modeling of class analogy (SIMCA); various pretreatments were investigated. For unfiltered honey, best SIMCA models gave correct classification rates of 95.5, 94.4, and 96% for the Irish, Mexican, and Spanish samples, respectively; PLS2 discriminant analysis produced a 100% correct classification for each of these honey classes. In the case of filtered honey, best SIMCA models produced correct classification rates of 91.7, 100, 100, and 96% for the Argentinean, Czech, Hungarian, and Irish samples, respectively, using the standard normal variate (SNV) data pretreatment. PLS2 discriminant analysis produced 96, 100, 100, and 100% correct classifications for the Argentinean, Czech, Hungarian, and Irish honey samples, respectively, using a second-derivative data pretreatment. Overall, while both SIMCA and PLS gave encouraging results, better correct classification rates were found using PLS regression.

KEYWORDS: Near-infrared; spectroscopy; honey; authenticity; geographical origin; classification

INTRODUCTION

Within the European Union (EU), honey is defined as "the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store, and leave in honeycombs to ripen and mature" (*1*). Honey is an extremely popular product; with its high glucose and fructose content, it is a source of energy that is instantly usable (*2*). It is widely appreciated as the only concentrated form of sugar available worldwide (*3*) and is also used as a food preservative (*4*). Adulteration of honey is economically advantageous, so its quality must be controlled analytically with the aim of guaranteeing authenticity and protecting the consumer from commercial exploitation (*5*). The rights of consumers and genuine food processors in terms of food adulteration and fraudulent or deceptive practices in food processing are set out in a European Union regulation regarding food safety and traceability (*6*). Under EU law, 16 types of honey are permitted to use a Protected Designation of Origin (PDO) label; 1 Greek, 2 Spanish, 2 French, 1 Italian, 1 Luxemburgian, and 9 Portuguese (*7*). Such honeys can demand a higher price than others, and in order to prevent fraudulent labeling of honey, a means of differentiating between honeys from different geographical locations must be devised.

Near-infrared spectroscopy is a technique that facilitates real-time measurements at all stages of processed food production from raw material analysis to ingredient and finished product verification. Spanning the wavelength range 780–2500 nm (*8*), NIR spectroscopy has been traditionally applied for the quantification of food compositional parameters, but it can also be used for the determination of complex quality properties such as texture and sensory attributes (*9, 10*). The development of instrumentation, measurement techniques, and chemometric applications which are relevant to the food industry have been widely reviewed in a number of recent publications (*9, 11–16*). NIR spectroscopy has been successfully applied in authentication studies of various food types, including differentiation of wines on the basis of grape variety (*17*) and olive oil on the basis of geographical origin (*18*).

In this paper, the use of NIR spectroscopy to confirm a geographical claim made about honey is examined.

MATERIALS AND METHODS

Sample Preparation. Artisanal unfiltered honey samples were collected directly from beekeepers from Ireland ($n = 88$), Mexico (n

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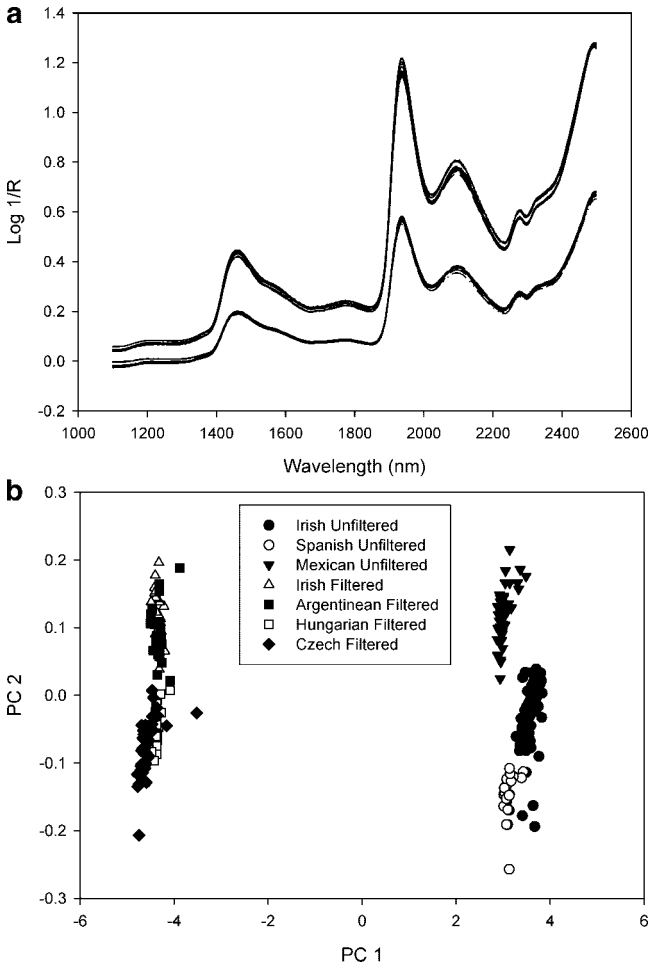

Figure 1. (**a**) NIR spectra of randomly selected filtered ($n = 19$) and unfiltered ($n = 24$) honeys (raw spectra; 1100–2498 nm wavelength range). **(b)** PCA scores plot showing separation between unfiltered and filtered honey samples (raw spectra; 1100–2498 nm wavelength range).

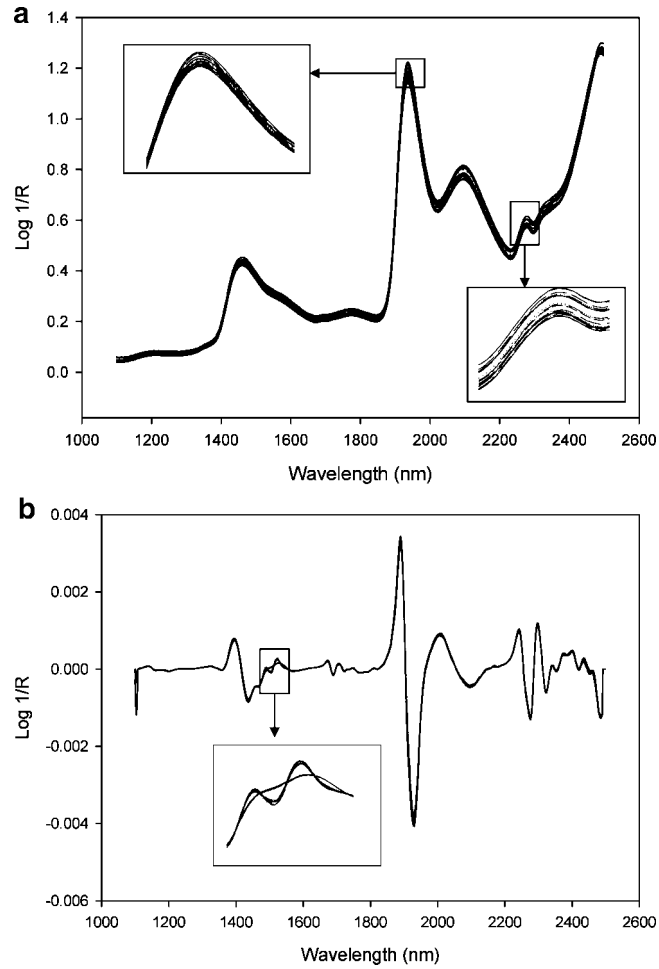

Figure 2. (**a**) NIR transflectance spectra (0.1 mm sample thickness) of 8 Irish, 8 Spanish, and 8 Mexican honey samples. (**b**) NIR transflectance spectra (0.1 mm sample thickness) of 8 Irish, 8 Spanish, and 8 Mexican honey samples after second-derivative pretreatment (9 data point gap).

$= 54$), and Spain ($n = 25$); these samples were scanned during 2004. Filtered honey samples were collected from beekeepers in Ireland ($n = 25$), Argentina ($n = 25$), the Czech Republic ($n = 50$), and Hungary ($n = 25$); filtered honeys were scanned during 2005. All honeys collected were stored at room temperature (18–25 °C) between collection and spectral acquisition (up to 12 months). Given that the honey samples were stored in the dark in screw-cap jars at moderate temperatures, it is unlikely that any significant change would have occurred during storage. However, because this application would be applied to honey samples of indeterminable age, such variability may increase the robustness of the discriminant models developed. Prior to spectral collection, honeys were incubated at 40 °C overnight in an air oven to dissolve any crystalline material, manually stirred to ensure homogeneity, and adjusted to a standard solids content (70° Brix) with distilled water. This standardization was designed to avoid adventitious classification on the basis of total solids content.

Spectral Collection. Honey samples (~50 mL) were placed in a covered beaker in a water bath held at 30 °C and allowed to equilibrate for 30 min before being scanned. Transflectance spectra were collected using a camlock cell and a gold-plated reflector (0.1 mm sample thickness; part no. 99213) on a scanning spectrophotometer (NIR Systems 6500, NIR Systems Inc., Silver Springs, MD). Between samples, the cell was cleaned using tepid distilled water and then Triton X-100 solution and finally rinsed with tepid distilled water before drying with a paper tissue; all washing liquids were equilibrated to 30 °C. Spectra were recorded in triplicate for each sample with the mean of these replicates being used in subsequent calculations. WINISI software (v 1.05; ISI International, Port Matilda, PA) was used for spectrophotometer control and spectral file manipulation.

Statistical Analysis. Raw spectra were exported from WINISI in JCAMP.DX format (*19*) and imported into The Unscrambler (v. 9.2; CAMO A/S, Oslo, Norway) for data analysis. A preliminary examination of the spectral data set for unusual or outlying samples was performed by principal component analysis (PCA). Classification techniques investigated in this work were discriminant partial least-squares regression (D-PLS2) and soft independent modeling of class analogy (SIMCA). For SIMCA analysis, PCA models were developed using every second sample from each individual country as a calibration set. Classification in SIMCA then used the remaining samples from the country in question and all of the other samples as a validation set. Class cutoff limits were set at the 5% level. For the D-PLS2, original PLS2 models were developed using 2/3 of the total sample set and were then tested using the remaining 1/3 of the samples. Dummy variables, one for each country involved, were generated and assigned a value of 0 or 1 for each of these variables; honey from the country being modeled was set equal to 1, and all other samples had this variable set to 0. For both PLS2 and SIMCA, full cross-validation was used in model development. First- and second-derivative pretreatments using the Savitzky–Golay (*20, 21*) method and the standard normal variate (SNV) transform (*22*) were investigated in all cases. All models were developed using the 1100–2498 nm wavelength region.

RESULTS AND DISCUSSION

First, spectra of the entire data set were plotted and examined; **Figure 1a** shows a representative subset. Spectra that are grouped at the higher absorbance on the plot are the unfiltered honey samples; spectra of the filtered honey samples exhibit

lower absorbance levels. Upon preliminary examination of the entire spectral set by principal component analysis, the filtered honey samples were clearly and completely separated from the unfiltered honeys (**Figure 1b**). The reasons for this separation may be that (i) differences actually exist between filtered and unfiltered honey spectra or (ii) instrumental drift over the period of the experimental data collection (1 year). Given that the instrument used in this study was not standardized, it is unsafe to assume that the spectral differences arise solely or even significantly from honey filtering; therefore, the two sample populations will be treated as separate for the purposes of this paper. Both unfiltered and filtered honey samples are found in the market place so it is desirable that classification models exist for both types. Within the two sample populations, some separation based on the geographical origin of the honey can be observed—for example, the Irish and Argentinean filtered honey lie together and somewhat apart from the Hungarian and Czech filtered honey. Equally, three separate groups representing the Irish, Spanish, and Mexican unfiltered honey samples can be seen on the right-hand side of the scores plot.

Study 1. Representative transflectance spectra of eight randomly selected Irish, Mexican, and Spanish honeys (n = 24) are shown in **Figure 2a**. At the peak located at 1936 nm some separation is observed between honey samples from different countries; the Spanish honeys have the lowest absorbance while the Irish and Mexican samples are overlapped. With regard to the peak at 2278 nm, the Irish samples have the highest absorbance with the Mexican and Spanish samples overlapped underneath them. The major features of these spectra are peaks at 1463 nm (OH, CH, and CH_2 deformations), 1936 nm (OH combinations), and 2096 and 2278 nm (CH combinations) (*23*). Transflectance spectra of aqueous solutions of fructose and glucose have been reported to contain absorbance peaks at almost identical locations (*13*). The second-derivative plot (**Figure 2b**) of these Irish, Mexican, and Spanish honey samples reveals greater spectral detail, with minima corresponding to the maxima in the original spectra. It is worth noting in this figure that there is a small section of the spectra between 1480 and 1560 nm where the Irish honey spectra can be seen to differ from the Mexican and Spanish spectra. The reason for this difference is unclear, but it is possible that this wavelength section may facilitate some subsequent separation between the Irish samples and the other samples.

Principal component analysis was carried out on the raw data to (a) assist with the detection of any outlying samples in the original data and (b) investigate any possible clustering of samples on the basis of geographic origin. A score plot for components 1 and 2 is shown in **Figure 3a**, and two main observations can be made from an examination of this plot. First, there are no obvious outlying samples; second, with the exception of two Spanish samples, honeys from each of the three countries have clustered closely together in ellipsoid patterns and at some distance from the others. PC1 chiefly effects a separation of Irish samples from those originating in the other two countries (Mexico and Spain); **Figure 3b** shows the loading plot associated with PC1 (which explains 87% of the variance in the spectral data set), and peaks at 1934 and 2098 nm demonstrate that these wavelengths play a major role in attaining this separation; structures absorbing at 1464 and 1522 nm also make a contribution. Similarly, PC2 (explaining 9% of spectral variance) separates Spanish and Mexican samples, and its loading plot (also seen in **Figure 3b**) shows that the main peaks which influence this separation are found at 1422 and 1928 nm.

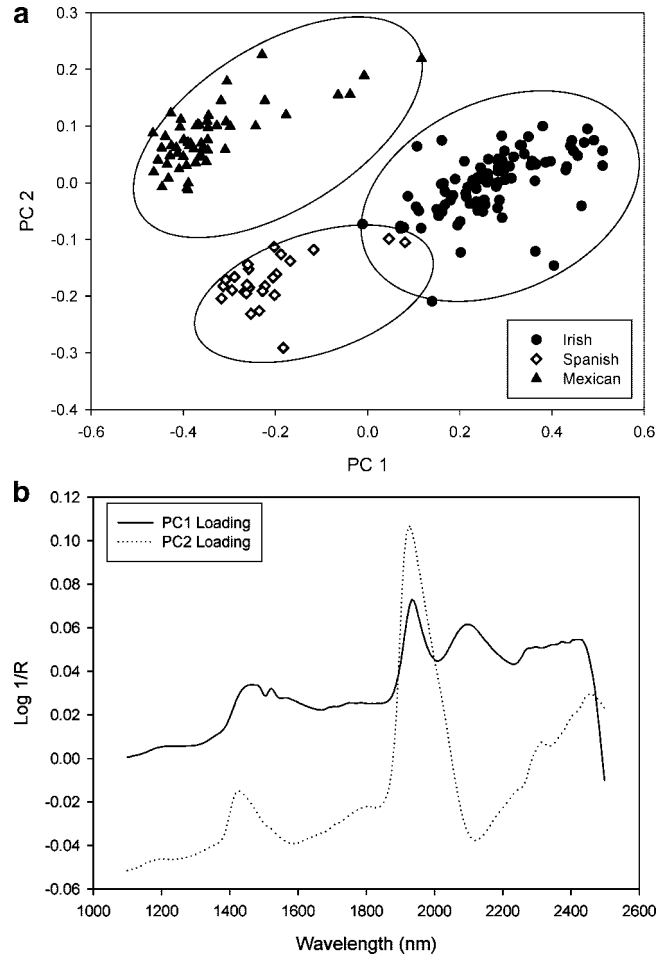

Figure 3. (**a**) PCA scores plot of Irish, Spanish, and Mexican honey samples (raw spectral data; 1100–2498 nm wavelength range). Ellipses drawn on this graph are for presentation purposes only. (**b**) *x*-loadings plot for PCA (raw spectral data; 1100–2498 nm wavelength range) for PC1 (explaining 87% of the variance) and PC2 (explaining 9% of the variance).

SIMCA Classification. In order to develop a classification model for honey from these countries, the spectral collection was subject to analysis by SIMCA. In the case of models produced using raw spectral data, 90.9, 92.6, and 96% correct classification rates were achieved for Irish, Mexican, and Spanish honey samples, respectively, on the prediction sample set. Significantly, none of these models produced any false positive results; i.e., no Mexican or Spanish honeys were wrongly classified as Irish, etc. The classification errors which did arise occurred because some samples from each country were not recognized as such; augmentation of the calibration sample set with larger numbers of samples may be expected to reduce this effect. This procedure was repeated using data that had been subjected to first- and second-derivative data pretreatment using gap sizes of 5, 9, 13, and 21 data points in each. In each case, the number of principal components used in the development of the models was either 3 or 4; the result for the Irish honey was consistent with the original findings using raw data, but those for Mexican and Spanish samples were poorer with lower correct classification rates and high levels of false positive identifications (**Table 1**). When false positives were observed for the Mexican honey model, these were Spanish honeys and vice versa. No Irish honeys were falsely classified as either Mexican or Spanish. The standard normal variate (SNV) transform was also applied to the raw spectral data;

Table 1. SIMCA Classification for Unfiltered Irish, Mexican, and Spanish Honey: Prediction Sample Set (1100–2498 nm Wavelength Range)

		Irish (n = 88)		Mexican (n = 54)		Spanish (n = 25)	
data pretreatment	#PC[b]	% correct classification	% false positives	% correct classification	% false positives	% correct classification	% false positives
raw data	4	90.9	0	92.6	0	**96**	**0**
1st deriv, 5 pts	3	88.6	0	92.6	48.1	96	76
1st deriv, 9 pts	3	88.6	0	92.6	46.3	96	76
1st deriv, 13 pts	3	88.6	0	92.6	46.3	96	72
1st deriv, 21 pts	3	87.5	0	90.7	46.3	96	72
2nd deriv, 5 pts	4	**95.5**	**0**	100	92.6	100	100
2nd deriv, 9 pts	4	90.1	0	98.2	57.4	96	96
2nd deriv, 13 pts	3	89.8	0	96.3	68.5	96	88
2nd deriv, 21 pts	3	89.8	0	94.4	50	96	84
SNV[a]	3	90.1	0	**94.4**	**0**	92	0

[a] SNV: standard normal variate. [b] #PC: number of principal components.

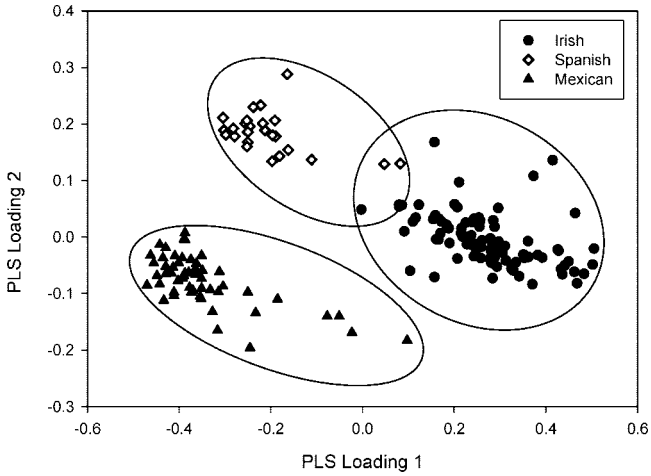

Figure 4. Discriminant PLS2 scores plot of Irish, Mexican, and Spanish honeys (raw spectral data; 1100–2498 nm). Ellipses drawn on this graph are for presentation purposes only.

results were similar to those obtained using raw data, with correct classification rates of 90.1, 94.4, and 92% for the Irish, Mexican, and Spanish models, respectively; no false positive classifications were found in any case. From **Table 1** it can be concluded that the best model for the classification of the Irish honey involved a second-derivative data pretreatment with 5 data point (10 nm) gap and the best model for prediction of Mexican honey involved SNV pretreatment of raw data while that for the prediction of Spanish honeys utilized unmodified spectra; these results are indicated in bold in the table.

Discriminant PLS2. The sample scores plot for the first and second loadings calculated following the application of D-PLS2 to the raw spectral data is shown in **Figure 4**. As was the case with the PCA scores plot described above, separation between the honey samples from the three countries was apparent; in fact, the PCA and D-PLS2 scores plots are very similar though inverted. As before, two Spanish honeys were located at some distance from the Spanish cluster and quite close to, if not inside, the cluster of Irish honey samples. The identity of these Spanish honeys was the same in both cases. Summary classification results for unmodified and pretreated spectral data following the application of D-PLS2 to the prediction set of samples are shown in **Table 2**. Honey from each of the countries was 100% correctly classified with no false positives in any case except for the second-derivative 5 data point model, in which one Spanish honey sample was misclassified in both the Spanish and the Mexican models. This performance shows the D-PLS2 approach to be superior to that reported above for SIMCA (**Table 1**). It is worth noting that the number of loadings used in each model was relatively low (n = 4, 6, or 8) with consequent positive implications for model robustness.

Study 2. As in study 1, no significant difference can be seen with the naked eye between the spectra of filtered honey samples from different geographic origins other than a small baseline offset. Following the application of PCA to the raw spectral data, one Argentinean sample was located some distance from the others in its cluster and had an associated large leverage in the PCA model; as a result, this single sample was excluded from the spectral data set before further analysis. Examination of this sample's spectrum alongside spectra of other Argentinean samples revealed obvious differences and justified its deletion from further data analysis.

SIMCA Classification. **Table 3** provides an overview of the results of SIMCA modeling; preferred models for each geographic origin are indicated in bold type. This selection was based on (a) the highest correct classification rate, (b) the lowest percentage of false positives, and (c) the smallest number of principal components in the model. In the case of honey from Argentina, the best model (first-derivative pretreatment, 21 data points) used four PCs and correctly classified 100% of Argentinean honeys but produced 8.3% false positive identifications; these false positives comprised entirely Irish samples. In the case of Czech and Hungarian honeys, preferred models correctly classified 100% of authentic honeys with no false positive classifications. These models were produced using a second-derivative (5 data points, 4 PCs) or SNV (5 PCs) pretreatment for the Czech honey model or unmodified spectral data (4 PCs) for the Hungarian honey model. The preferred model for Irish honey (first-derivative, 9 data point pretreatment, 4 PCs) correctly classified 96% of Irish honeys but had an associated false positive classification rate of 12%; these samples comprised entirely Argentinean honeys.

Discriminant PLS2. A D-PLS2 investigation was carried out on the entire filtered data set as previously described for unfiltered honey. As can be seen from **Table 4**, the results are generally very good for the Czech and Hungarian honey samples, but the same problem of misclassification between Irish and Argentinean honey samples that existed in the SIMCA model persisted here. The best models (bold type in **Table 4**) for the prediction of the Irish honey samples used a first-derivative data pretreatment while for the Argentinean honey prediction, the best model used an SNV (standard normal variate) data pretreatment; the Irish model gave a 100% correct classification with a 25% false positive result while the Argentinean model gave a 75% correct classification with no false positives. All false positives for the Irish model were Argentinean honeys incorrectly classified as Irish, and all false

Table 2. D-PLS2 Classification for Unfiltered Irish, Mexican, and Spanish Honey Prediction Samples (1100–2498 nm Wavelength Range)

		Irish ($n = 29$)		Mexican ($n = 18$)		Spanish ($n = 18$)	
data pretreatment	#L[b]	% correct classification	% false positives	% correct classification	% false positives	% correct classification	% false positives
raw data	4	100	0	100	0	100	0
1st deriv, 5 pts	6	100	0	100	0	100	0
1st deriv, 9 pts	6	100	0	100	0	100	0
1st deriv, 13 pts	6	100	0	100	0	100	0
1st deriv, 21 pts	6	100	0	100	0	100	0
2nd deriv, 5 pts	6	100	0	100	2.7	87.5	0
2nd deriv, 9 pts	8	100	0	100	0	100	0
2nd deriv, 13 pts	8	100	0	100	0	100	0
2nd deriv, 21 pts	8	100	0	100	0	100	0
SNV[a]	4	100	0	100	0	100	0

[a] SNV: standard normal variate. [b] L: number of PLS loadings.

Table 3. SIMCA Classification for Filtered Argentinean, Czech, Hungarian, and Irish Honey: Prediction Sample Set (1100–2498 nm Wavelength Range)

		Argentinean ($n = 25$)		Czech ($n = 50$)		Hungarian ($n = 25$)		Irish ($n = 25$)	
data pretreatment	#PC[b]	% correct classification	% false positives	% correct classification	% false positives	% correct classification	% false positives	% correct classification	% false positives
raw data	4	100	84	90	0	100	0	96	96
1st deriv, 5 pts	4	88	60	98	0	100	0	96	56
1st deriv, 9 pts	4	88	52	98	0	100	0	**96**	**12**
1st deriv, 13 pts	4	100	20.8	98	0	100	0	96	76
1st deriv, 21 pts	4	**100**	**8.3**	98	0	100	0	96	76
2nd deriv, 5 pts	4	100	91.7	**100**	**0**	100	24	100	100
2nd deriv, 9 pts	4	88	80	98	0	100	0	96	96
2nd deriv, 13 pts	5	91.7	66.7	98	0	100	0	96	68
2nd deriv, 21 pts	5	91.7	20.8	98	0	100	0	96	48
SNV[a]	5	91.7	16.7	**100**	**0**	100	0	96	20

[a] SNV: standard normal variate. [b] #PC: number of principal components.

Table 4. D-PLS2 Classification for Filtered Argentinean, Czech, Hungarian, and Irish Honey Samples: One-Third of Full Sample Set Used for Prediction (1100–2498 nm Wavelength Range)

		Argentinean ($n = 8$)		Czech ($n = 16$)		Hungarian ($n = 8$)		Irish ($n = 8$)	
data pretreatment	#L[b]	% correct classification	% false positives	% correct classification	% false positives	% correct classification	% false positives	% correct classification	% false positives
raw data	4	50	0	100	0	100	0	75	37.5
1st deriv, 5 pts	5	37.5	0	100	0	100	0	**100**	**25**
1st deriv, 9 pts	5	37.5	0	100	0	100	0	**100**	**25**
1st deriv, 13 pts	5	37.5	0	100	0	100	0	**100**	**25**
1st deriv, 21 pts	5	25	0	100	0	100	0	**100**	**25**
2nd deriv, 5 pts	5	62.5	0	100	0	100	0	87.5	50
2nd deriv, 9 pts	5	62.5	0	100	0	100	0	87.5	50
2nd deriv, 13 pts	5	50	0	100	0	100	0	100	37.5
2nd deriv, 21 pts	5	50	0	100	0	100	0	100	37.5
SNV[a]	5	**75**	**0**	100	0	100	0	87.5	75

[a] SNV: standard normal variate. [b] #L: number of PLS loadings.

positives for the Argentinean model were Irish honeys incorrectly classified as Argentinean. All models gave a 100% correct classification for the Czech and the Hungarian honey samples with no false positives.

Given that the only confusion between honeys from different geographic origins arose between samples from Ireland and Argentina, the possibility of discriminating between these two sources alone was investigated using D-PLS1. The original PLS1 models were constructed using 2/3 of the total Irish and Argentinean sample set, and the remaining 1/3 of the samples was used to test the models (8 Irish and 8 Argentinean samples); although this is a relatively small number of samples, results are very encouraging and can be seen in **Table 5**. The only model that does not show 100% correct discrimination between the Irish and Argentinean samples is the raw data model; all of the models constructed using first or second derivative or SNV data pretreatment show a 100% correct classification of both the Irish and Argentinean samples. **Figure 5** shows the prediction plot for the Argentinean and Irish samples only while **Figure 6** shows loadings 1 and 2 for this D-PLS1 regression; loading 1 accounts for 76% and loading 2 accounts for 13% of the explained *x*-variance. It can be concluded from **Figure 6** that wavelength regions at 1420–1434 and 1900 nm (OH, CH, and CH_2 deformations) and 1960 nm (OH combinations) (*23*) contribute particularly strongly to the model development. In practice, a hierarchical analysis could be effectively applied to this problem. The near-infrared signal from an unknown filtered honey sample claiming to originate in one of the four countries could be analyzed initially using either the SIMCA or D-PLS2 models developed in this work; if this analysis showed the sample to be Czech or Hungarian, this identification could be accepted with a high level of certainty. If however the sample

Table 5. D-PLS1 Classification for Filtered Argentinean and Irish Honey Samples: One-Third of Full Sample Set Used for Prediction (1100–2498 nm Wavelength Range)

		Argentinean ($n = 8$)		Irish ($n = 8$)	
data pretreatment	#L[b]	% correct classification	% false positives	% correct classification	% false positives
raw data	5	87.5	0	100	12.5
1st deriv, 5 pts	4	100	0	100	0
1st deriv, 9 pts	4	100	0	100	0
1st deriv, 13 pts	4	100	0	100	0
1st deriv, 21 pts	4	100	0	100	0
2nd deriv, 5 pts	4	100	0	100	0
2nd deriv, 9 pts	4	100	0	100	0
2nd deriv, 13 pts	4	100	0	100	0
2nd deriv, 21 pts	4	100	0	100	0
SNV[a]	4	100	0	100	0

[a] SNV: standard normal variate. [b] #L: number of PLS loadings.

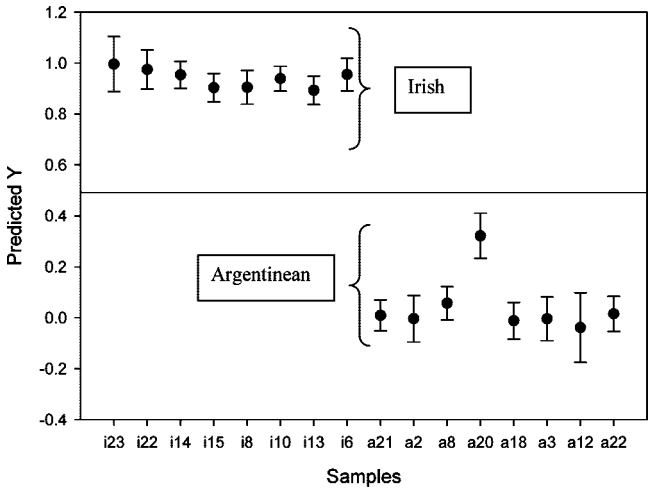

Figure 5. Discriminant PLS1 prediction plot of Irish and Argentinean honey samples (first derivative, 13 points).

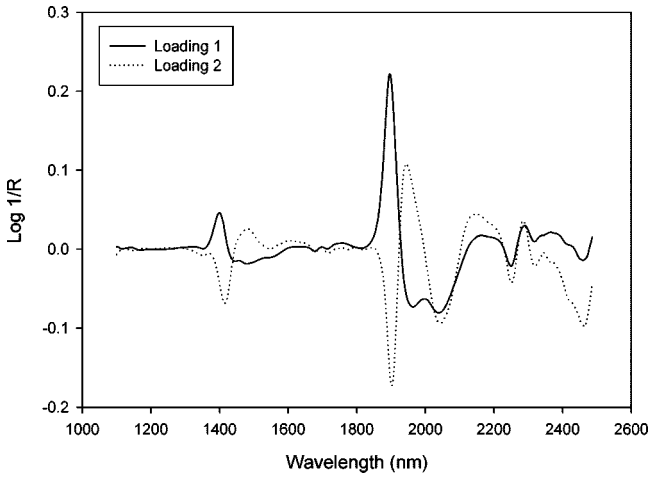

Figure 6. Plot of loadings 1 and 2 for Argentinean vs Irish discriminant PLS1 model.

was predicted to be from Argentina or Ireland, then the D-PLS1 model could be applied to positively identify which of those two countries the honey originated in.

On the basis of results outlined in this paper, it can be concluded that honey samples, at least from the countries investigated, can be correctly classified using NIR spectroscopy with a high degree of certainty. Problems may arise however if a honey sample from a country which has not been included in this work is analyzed; in this respect, the SIMCA models may offer some greater security than the discriminant approaches given that their basis is the modeling of similarity rather than difference. Translation of this technique into an industrial setting would require the establishment of a large, authentic sample database at significant expense; given the results reported in this paper, however, the implementation of an NIR-based provenance confirmation strategy may be justified.

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