https://doi.org/10.48196/019.02.2023.04

Submitted: December 11, 2023
Accepted: December 21, 2023

Short-Wave Near-Infrared Spectroscopy and Chemometrics to Detect Adulteration of Honey with Sucrose Syrup

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ABSTRACT

Near-infrared spectroscopy (NIRS) coupled with multivariate statistical analysis was assessed as a rapid method of detecting honey adulteration in the Philippines. The sample set contained 288 spectra of authentic and adulterated honey samples obtained from six farms in the provinces of Benguet and La Union. Spectral data were pre-processed using ParLeS software in which partial least squares regression (PLSR) analysis was employed. Multivariate analyses like PLSR, principal component analysis (PCA) and linear discriminant analysis (LDA) were performed to predict the level of adulteration, reducing sugar content, and apparent sucrose content. Calibration models for predicting adulteration level (from none to highly adulterated) gave the best results using PLSR on spectral data without pretreatment (calibration R2 > 0.93, validation R2 > 0.94). Principal components (PCs) of the spectral data were extracted using PCA wherein the NIR absorbance bands for sugar (836-840 nm and 978-980 nm) and water (938-940 nm, 978-980 nm, 984-986 nm and 992-994 nm) in honey were identified. Using LDA, calibration models were able to classify honey based on level of adulteration and apparent sucrose; overall accuracies of 99.5% and 100% were observed, respectively. When validated, however, the LDA models could not detect pure honey (out of 6 and 11 samples, respectively), while 86.7% and 100% of adulterated honey could be detected, respectively. Further sampling is recommended to strengthen LDA models.

Keywords: *near-infrared, spectroscopy, honey, adulteration, detection, chemometrics*

INTRODUCTION

Honey is a natural substance produced by honey bees and is widely used as a sweetener and in traditional medicine. Global demand has been increasing over the years, but production has remained relatively constant except for China where production volume doubled in the period 1993-2013 (Ulberth, 2016). Due to high demand and inadequate production, honey has become one of the most common targets for economically motivated adulteration (EMA), also known as food fraud (Moore et al., 2012). The price per unit of honey in the UK in 2023 was more than 13 times higher than the price of sugar (Statista, 2023); this price difference is a strong driver for EMA, and is projected to increase in the next 5 years (Statista, 2023). International standards require that honey must be free of organic or inorganic materials that are not part of its natural composition (CAC, 2022; European Commission, 2002). However, EMA of honey includes dilution or the use of extenders, supplemental feeding of sugar syrups to bees to increase production, the use of unapproved or inappropriate drugs and chemicals on bees, and hiding the true country of origin to avoid fees, testing or product bans. An example of the latter practice occurred between March 2002 and April 2008, when honey originating from China was misdeclared as coming from Russia, India and several Asian countries, including the Philippines. This scheme was devised to avoid anti-dumping fees close to US$80 million (Strayer et al., 2014). A literature review by Fakhlaei et al. (2020) showed that consuming fraudulent products also has negative effects on human health, since this can lead to diabetes, obesity, and high blood pressure. In-vivo studies (human or animal subjects) also show negative effects of adulterants on internal organs such as the stomach, liver, and kidney.

The major components of honey include moisture (20 g per 100 g), fructose (37-39 g per 100 g), glucose (30-32 g per 100 g), sucrose (0.5-2.0 g per 100 g) and ash (0.2 g per 100 g). The ratio of fructose to glucose is generally 1.2 : 1.0. Minor components include proteins, organic acids, vitamins and minerals, and phenolic and volatile compounds. The proportions of these compounds in honey are greatly dependent on the nectar composition of flora accessible to bees. These in turn are affected by variables such as species, climate, geographic location, and pollution levels. The presence of numerous factors affecting honey quality complicates efforts to identify a set of markers for classification of unknown products. However, the presence of additives such as sugar syrups can alter the profile of honey products and can serve as an indicator of adulteration (Ulberth, 2016; da Silva et al., 2016). According to Codex Alimentarius standards for honey, moisture content must be less than 20%, reducing sugars higher than 60%, and sucrose levels below 5% (CAC, 2022). Brazilian honey samples analysed by Azeredo et al. (2003) had the following physico-chemical properties: 18.59 – 19.58% moisture content, 62.6 – 69.1% reducing sugars, 3.5 – 5.4% sucrose, and pH of 3.10 – 4.05.

There are several simple methods claimed to detect adulteration in honey, such as the *flame test* (matchstick dipped in pure honey will still burn), *water test* (adulterated honey will quickly dissolve), and the blot test (adulterated honey on white cloth or paper will soak through). However, these tests can be inconsistent, and there are laboratory methods that are more reliable (Honeys.PH, 2023). Authentication techniques for detecting honey adulteration include thin-layer chromatography, carbon isotopic analysis, gas chromatography, anion exchange chromatography, infrared-based spectroscopy, nuclear magnetic resonance, Raman spectroscopy and mass spectrometry. Many of these techniques require costly instruments, significant analytical skill, and challenging sample pre-treatment and extraction methods. In comparison, infrared (IR)-based analysis, such as those based on the near- and mid-IR ranges, provide rapid results with minimal pre-processing of samples (Wu et al., 2017; Li et al., 2017). Near-infrared spectroscopy (NIRS), in particular, has found numerous applications in the food industry. Analysis is non-destructive and can be easily combined with chemometric methods of analysis (both qualitative and quantitative) (Cen and He, 2007). NIRS has been used to detect adulteration in South African

honey (860-2500 nm) using three different instruments; overall accuracy of classification was above 93% (Guelpa et al., 2017). A similar effort is needed to test honey products in the Philippines which are widely sold but are known to be adulterated with artificial sweeteners. Data presented by Micor (2013) showed that 8 of 14 (57%) locally available honey and 7 of 25 (28%) imported brands were adulterated. The general objective of the present study was to develop NIRS-based models for rapid estimation of adulteration levels in Philippine honey

METHODOLOGY

Honey Samples

Typical *Apis mellifera* honey samples were collected from six different apiaries (coded as Samples A, B, C, D, E and F) on the main Philippine island of Luzon; all the apiaries were located in Baguio City (in Benguet province) or La Union province. Approximately 500 g of honey were collected from each apiary from December 2013 to February 2014; samples were stored at room temperature. Moisture content and °Brix of honey samples were determined using an Atago digital refractometer.

Preparation of Adulterated Honey

Sugarcane syrup was used as an adulterant and was prepared as a mixture of table sugar, distilled water, citric acid and cream of tartar; proportions of each component in the mixture were obtained from interviews with honey producers and could not be disclosed. From an initial Brix value of 64°, the mixture was heated at 90-100°C until the final Brix was approximately 70°.

Undiluted honey samples had the following properties: 79-80°Brix, 20-21% MC and 3.0-4.0 pH level. Samples were standardized to 70°Brix by gradual addition of distilled water to minimize the effect of natural variation in soluble solids of samples and avoid false results (Kelly et al., 2004, Zhu et al., 2010, Rios-Corripio et al., 2012, Guelpa et al., 2017); samples were heated to 35°C to dissolve any crystals present.

For each pure honey sample, sixteen 10-mL dilutions were prepared in test tubes from 0% to 30% (v/v) in increments of 2%; three replicate sets of dilutions were prepared. Diluted samples were incubated at 50°C using a water bath for 30 min, followed by homogenization using a vortex mixer at low speed. Diluted samples were allowed to equilibrate to room temperature for 4 hours prior to NIR spectroscopic analysis. A total of 288 spectra were randomly divided into calibration (192 samples) and validation sets (96 samples).

NIR Spectral Acquisition, Pretreatment and Analysis

Spectral acquisition was performed using instruments, fiber optics and software from Ocean Optics Inc (Florida, USA); these included a USB-4000-VIS-NIR spectrometer (operating range of 300-1000 nm) with Spectrasuite software, QR-200-7-VIS-BX fiber optic cables, CUV-UV cuvette holder (10-mm pathlength), and a HL-2000 tungsten-halogen lamp (360-2400 nm). The system was allowed to warm up for 60 min prior to data acquisition. Spectral data for each dilution represented the average of 40 scans; integration time was set at 7 msec. Absorption spectra of diluted samples was generated using distilled water as a reference; dark spectra was acquired with the light

Table 1. Pre-processing methods for spectral data[2]

Pretreatment	Description
Mean centering (MC)	First stage in pre-processing; the average value of each spectra is subtracted from each variable of the spectra.
Multiplicative scatter correction (MSC)	To reduce effect of non-uniform light scattering; degree of light scatter is affected by radiation wavelength, particle size, and refractive index of the material.
Standard normal variate (SNV)	Sample spectra is normalized to a mean of zero and a variance equal to 1. Eliminates interference of light scatter, and effect of particle size and light distance.
Savitzky-Golay (SG) smoothing	To reduce random noise from spectra
First derivative (ID) and second derivative (2D) transformation	Used to increase spectral resolution, remove baseline shifts and background effects, and resolve superimposed peaks.

[2]*Source: Viscarra-Rossel (2008); Nicolai et al (2007); Reich (2005)*

source turned off. Only absorption spectra in the NIR range of 700-1000 nm was used for analysis.

Different combinations of various pre-processing techniques (**Table 1**) were used to improve calibration models for estimating adulteration levels in terms of (a) light scattering and baseline correction to normalize spectra, (b) de-noising and smoothing to improve the signal-to-noise ratio and reduce the effect of random noise, and (c) first-derivative (1D) differentiation to enhance spectral resolution. ParLeS software was used for pre-processing the spectral data; details on the pre-processing capabilities of ParLeS are described by Viscarra-Rosel (2008). The best combinations were selected by generating calibration models using the partial least squares regression (PLSR) option of ParLeS. For each pure honey sample, the spectral data of the three replicate sets of dilutions were randomly divided into separate calibration and validation sets at a 2:1 ratio (similar to Chen et al., 2011). Spectral data of all honey samples was also analysed as a combined set in the same manner. For the calibration set, PLSR with cross-validation was carried out using the leave-one-out option. Selection of the best calibration models was based on values of R^2 and the ratio of performance to deviation (RPD) as generated by ParLeS; interpretation of R^2 and RPD values is given in **Tables 2** and **3**.

Pre-processed spectral data at 700-1000 nm (2-nm intervals) was also subjected to principal component analysis (PCA) to describe variations in spectra using synthetic variables generated as a linear combination of the original data. These synthetic variables, or principal components (PC), are not correlated to each other, and therefore, multi-collinearity is avoided (Oliveri and Forina, 2012). The number of PCs was determined by retaining only those with eigenvalues greater than one. Linear discriminant analysis

Table 2. Guidelines for the interpretation of coefficients of correlation (R) and determination (R^2)[z]

R	R^2	Interpretation
Up to ± 0.5	<0.25	Not usable in calibration
± 0.51 - 0.70	0.26 - 0.49	Poor correlation, needs further
± 0.71 - 0.80	0.50-0.64	Usable for rough screening
±0.81-0.90	0.66-0.81	Suitable for screening and other
± 0.91 - 0.95	0.83 - 0.90	Can be used with caution in most
± 0.96 - 0.98	0.92-0.96	Can be used in most applications,
± 0.99 or higher	Higher than 0.98	Excellent, can be used in any application

[z]*Source: Williams (2001)*

Table 3. Guidelines for interpreting relative percentage deviation (RPD)[z]

RPD	Classification	Application
0.0-2.3	Very poor	Not recommended for use
2.4-3.0	Poor	Very rough screening
3.1-4.9	Fair	Screening
5.0-6.4	Good	Quality control
6.5-8.0	Very good	Process control
8.1 or higher	Excellent	Any application

[z]*Source: Williams (2001)*

Table 4. Physico-chemical properties[z] of *Apis mellifera* honey from Northern Luzon, Philippines

Honey Sample	% MC	°Brix	pH	%TS	%RS	% AS
A	18.0	81.5	3.6	81.5	69.5	12.0
B	18.4	80.0	3.6	79.4	69.3	10.1
C	19.0	79.1	3.8	77.3	65.3	12.0
D	18.0	80.5	3.4	72.6	68.3	4.3
E	18.5	79.7	3.7	76.4	62.3	14.1
F	18.3	80.0	3.6	79.8	65.7	14.1
Average	18.4	80.1	3.6	77.8	66.7	11.1
Published data[y]	17.1	-	3.8	68.5	64.4	0.4
	-	65.7-79.6	—	—	60.7-81.8	—
	19.2	-	3.6	-	66.2	4.5

[z]*For each honey sample, values represent the mean of three replicate samples; MC - moisture content, TS - total sugars, RS - reducing sugars, AS - apparent sucrose*

Source: [y]Yucel and Sultanoglu (2013); Li et al (2017); Azeredo et al (2003)

(LDA) was then used to analyze PCs to classify diluted honey samples according to their level of apparent sucrose and degree of adulteration.

PCA and LDA were performed using the Microsoft Excel XLSTAT add-in to detect adulteration based on sucrose level, and to predict degree of adulteration based on dilution level. From the total collection of samples, 192 and 96 honey samples were randomly selected as calibration and validation sets, respectively. Samples with apparent sucrose higher than 6% were considered as adulterated (CAC, 2022); samples with lower levels were designated as authentic. Honey samples with dilution levels of 0%, 1-9%, 10 -19%, and 20-30% were considered as pure, low-level adulterated, medium-level adulterated, and high-level adulterated samples, respectively.

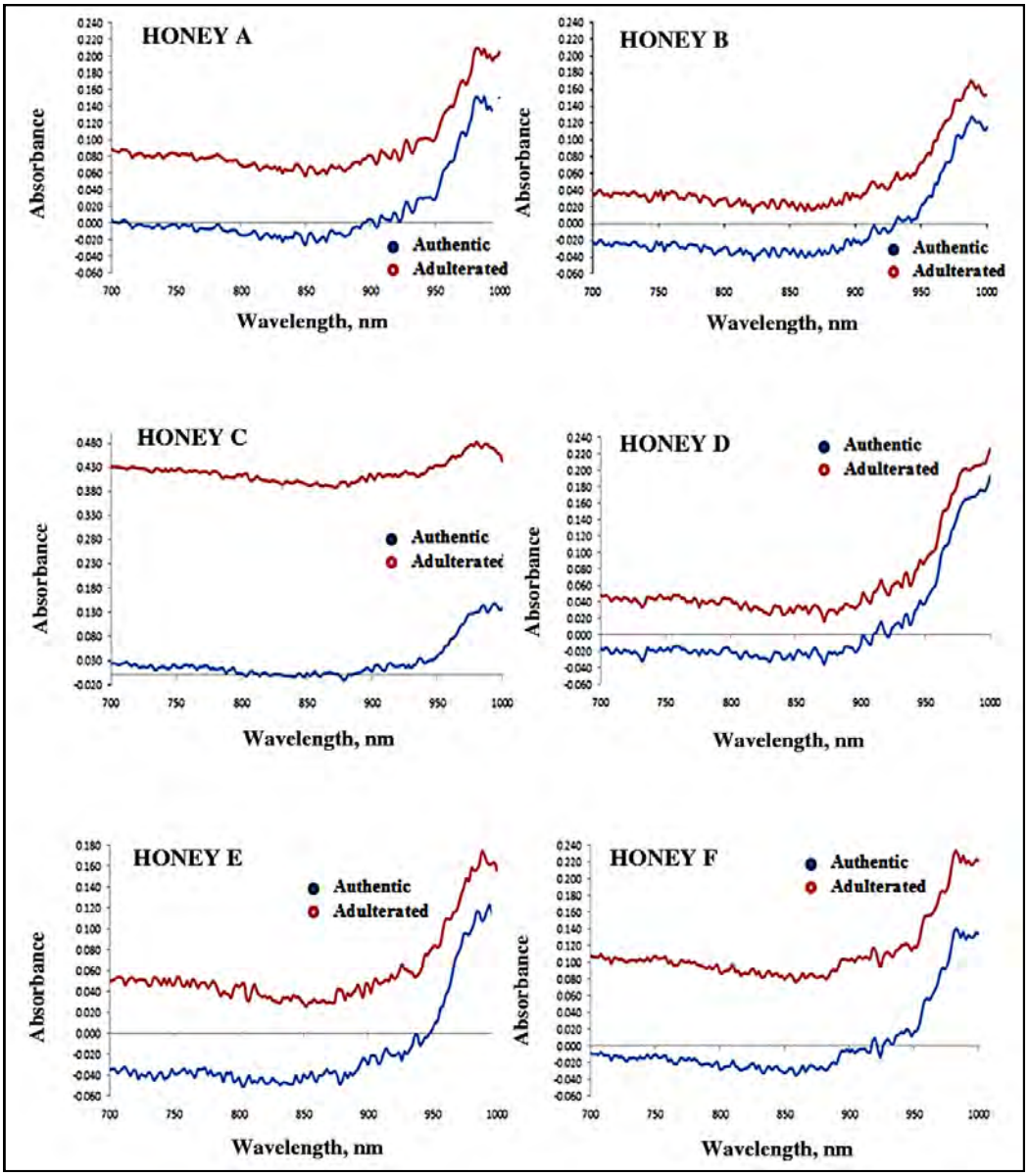

Figure 1. Comparison of representative near-infrared spectra (700-1000 nm) of authentic and adulterated honey samples adjusted to 70° Brix

Measurement of Sugar Levels

Standard wet chemistry methods for measuring total sugars (TS) and reducing sugars (RS) were used for developing NIR calibration models and for validation. The phenol sulfuric method described by Albalashmeh et al. (2013) was used for determining TS levels, while the dinitrosalicylic (DNS) method described by Goncalves et al. (2010) was used for measuring RS in adulterated honey samples. For TS and RS, absorbance was measured at 490 nm and 540 nm, respectively, using a UV-VIS spectrophotometer. Apparent sucrose was calculated as the difference between TS and RS.

RESULTS AND DISCUSSION

Physico-chemical Properties and Pollen Content of Honey Samples

Based on standards of the Codex Alimentarius Commission (CAC), moisture content of honey should not exceed 20%, reducing sugars should not be less than 60%, and sucrose content not greater than 5% (CAC, 2022). MC is dependent on the harvest season and local weather conditions; studies of honey from different countries by Azeredo et al., (2003), Escuredo et al., (2013) and Yücel and Sultanoğlu (2013) showed MC ranges of 18.6-19.6% (Brazil), 16.9-18.0% (northwest Spain) and 14.0-20.8% (Hatay, Turkey), respectively. Level of RS is also affected by climate, as well as flower types (Tornuk et al., 2013); on the other hand, sucrose levels are an important indicator of honey maturity and adulteration. High levels of sucrose may be a sign of adulteration with inexpensive sweeteners, early harvesting, or extended feeding of bees with sucrose syrup (Escuredo et al., 2013). **Table 4** shows the physico-chemical properties of honey gathered from the sampling sites; published data is included for comparison. Results show that all the honey samples pass the CAC (2022) standards for MC (less than 20%) and RS (higher than 60%). However, in terms of apparent sucrose, all the samples except Sample D showed excessive levels.

Table 5. Partial least squares regression to predict level of adulteration of individual honey samples using near-infrared spectral range of 700-1000 nm

Sample	Spectral Pretreatment[z]	No. of Factors	Calibration Set[y]			Validation Set		
			R^2	RMSE	RPD	R^2	RMSE	RPD
A	None	4	0.972	1.59	6.03	0.976	1.66	5.82
	SG	4	0.973	1.56	6.15	0.978	1.65	5.86
	SG-MC	4	0.972	1.57	6.10	0.975	1.79	5.40
	SG-1D-MC	10	0.958	1.96	4.89	0.963	2.64	3.67
B	None	4	0.931	2.40	3.85	0.942	2.20	4.01
	SG	4	0.945	2.15	4.30	0.941	2.16	4.09
	SG-MC	3	0.952	2.00	4.62	0.951	1.89	4.67
	SG-1D-MC	2	0.835	3.70	2.50	0.918	2.50	3.52
C	None	5	0.985	1.11	8.29	0.970	1.73	5.26
	SG	5	0.983	1.19	7.75	0.962	1.84	4.97
	SG-MC	5	0.985	1.09	8.41	0.973	1.55	5.89
	SG-1D-MC	5	0.988	0.99	9.27	0.978	1.48	6.18
D	None	4	0.958	1.87	4.95	0.962	2.64	3.56
	SG	4	0.949	2.04	4.52	0.947	3.17	2.95
	SG-MC	4	0.947	2.10	4.39	0.944	3.26	2.88
	SG-1D-MC	5	0.940	2.21	4.17	0.940	2.36	3.97
E	None	4	0.915	2.52	3.49	0.987	2.05	4.53
	SG	3	0.927	2.34	3.75	0.980	2.35	3.95
	SG-MC	2	0.939	2.14	4.12	0.975	2.33	3.99
	SG-1D-MC	2	0.912	2.56	3.44	0.959	3.34	2.78
F	None	4	0.968	1.66	5.68	0.967	1.72	5.39
	SG	4	0.961	1.84	5.14	0.964	1.74	5.33
	SG-MC	3	0.959	1.88	5.02	0.960	1.81	5.12
	SG-1D-MC	2	0.965	1.74	5.42	0.964	1.79	5.17

[z]SG - Savitzky-Golay; MC - mean centering; ID - 1st derivative
[y]With leave-one-out cross validation; RMSE - root mean square error; RPD - ratio of performance to deviation

Partial Least Squares Regression Models

Figure 1 shows the NIR spectra of representative honey samples that were pure or adulterated. As adulteration level increased, absorbance values also increased regardless of the source. Sample C showed the greatest increase in absorbance among the six samples, possibly due to differences in the profile of honey sources.

Results of PLSR for predicting level of adulteration, reducing sugar level, and apparent sucrose level are shown in **Table 5, 6** and **7**, respectively. The best results were obtained for predicting adulteration level in the wavelength range of 700-1000 nm, with R^2 in the range of 0.835 – 0.988 and 0.918 – 0.987 for the calibration and validation sets, respectively (**Table 5**). Furthermore, calibration models using spectral data without any pretreatment still obtained $R^2 > 0.930$; when validated, R^2 for all samples was higher than 0.940. Recommended applications based

Table 6. Partial least squares regression to predict reducing sugars of individual honey samples using near-infrared spectral range of 700-1000 nm.

Sample	Spectral Pretreat-ment[z]	No. of Factors	Calibration Set[y]			Validation Set		
			R^2	RMSE	RPD	R^2	RMSE	RPD
A	None	2	0.892	1.577	3.09	0.955	1.087	4.43
	SG	3	0.884	1.647	2.96	0.917	1.497	3.21
	SG-MC	2	0.908	1.450	3.36	0.914	1.416	3.40
	SG-1D-MC	1	0.903	1.491	3.27	0.887	1.758	2.74
B	None	3	0.748	2.454	2.03	0.709	2.464	1.76
	SG	3	0.733	2.507	1.98	0.708	2.386	1.82
	SG-MC	3	0.726	2.563	1.94	0.740	2.266	1.91
	SG-1D-MC	2	0.630	2.990	1.66	0.662	2.795	1.55
C	None	7	0.626	2.831	1.64	0.531	3.112	1.44
	SG	4	0.668	2.633	1.77	0.707	2.379	1.89
	SG-MC	6	0.694	2.555	1.82	0.633	2.747	1.64
	SG-1D-MC	2	0.613	2.877	1.62	0.751	2.211	2.03
D	None	2	0.590	2.936	1.58	0.770	2.570	1.81
	SG	2	0.601	2.895	1.60	0.778	2.502	1.85
	SG-MC	4	0.645	2.734	1.69	0.690	2.919	1.59
	SG-1D-MC	2	0.609	2.861	1.62	0.749	2.587	1.79
E	None	2	0.693	2.362	1.83	0.810	1.769	2.27
	SG	2	0.694	2.363	1.83	0.833	1.641	2.45
	SG-MC	2	0.691	2.372	1.82	0.865	1.499	2.68
	SG-1D-MC	2	0.620	2.642	1.64	0.832	1.913	2.10
F	None	3	0.779	2.127	2.15	0.689	2.310	1.68
	SG	3	0.775	2.148	2.13	0.728	2.144	1.81
	SG-MC	1	0.815	1.938	2.36	0.823	1.667	2.33
	SG-1D-MC	1	0.805	1.989	2.30	0.843	1.599	2.43

[z]*SG - Savitzky-Golay; MC - mean centering; 1D - 1st derivative*
[y]*With leave-one-out cross validation; RMSE - root mean square error; RPD - ratio of performance to deviation*

Table 7. Partial least squares regression to predict apparent sucrose content of individual honey samples using near-infrared spectral range of 700-1000 nm.

Sample	Spectral Pretreatment[z]	No. of Factors	Calibration Set[y]			Validation Set		
			R^2	RMSE	RPD	R^2	RMSE	RPD
A	None	3	0.863	1.770	2.74	0.852	1.960	2.38
	SG	3	0.868	1.737	2.80	0.857	1.900	2.46
	SG-MC	2	0.870	1.722	2.82	0.881	1.634	2.86
	SG-1D-MC	2	0.875	1.687	2.88	0.872	1.840	2.54
B	None	2	0.696	2.481	1.64	0.635	2.761	1.52
	SG	2	0.699	2.758	1.85	0.634	2.780	1.51
	SG-MC	1	0.714	2.210	1.90	0.637	2.725	1.54
	SG-1D-MC	1	0.724	2.742	1.94	0.663	2.644	1.59
C	None	3	0.586	2.882	1.56	0.716	1.989	1.84
	SG	1	0.624	4.011	1.12	0.754	3.291	1.11
	SG-MC	6	0.726	2.336	1.92	0.526	2.951	1.24
	SG-1D-MC	4	0.608	2.795	1.61	0.732	1.915	1.92
D	None	2	0.676	2.729	1.79	0.714	2.706	1.71
	SG	2	0.676	2.730	1.79	0.712	2.719	1.70
	SG-MC	3	0.668	2.788	1.75	0.666	3.187	1.45
	SG-1D-MC	2	0.675	2.737	1.78	0.722	2.703	1.71
E	None	6	0.688	2.495	1.80	0.756	2.507	1.56
	SG	3	0.657	2.594	1.73	0.849	1.761	2.22
	SG-MC	2	0.656	2.597	1.73	0.863	1.553	2.51
	SG-1D-MC	2	0.652	2.622	1.71	0.825	2.298	1.70
F	None	1	0.851	2.024	2.42	0.739	2.687	1.32
	SG	1	0.851	2.220	2.21	0.739	2.985	1.19
	SG-MC	1	0.836	1.958	2.50	0.743	2.199	1.61
	SG-1D-MC	2	0.848	1.880	2.61	0.679	2.404	1.48

[z] *SG - Savitzky-Golay; MC - mean centering; 1D - 1st derivative*

[y] *With leave-one-out cross validation; RMSE - root mean square error; RPD - ratio of performance to deviation*

on RPD values for spectra without pretreatment showed that calibration models could be used for screening samples B, D, and E, while models for samples A, C, and F could be used for higher-level applications such as quality control or process control. The number of factors for PLSR models was 5 or less, except for sample A honey spectra pretreated using SG-1D-MC which needed 10 factors.

In comparison, models for reducing sugars and apparent sucrose were less consistent across samples. For reducing sugars, calibration R^2 for sample A honey had a range of 0.884 – 0.908, while calibration R^2 for sample D honey was 0.590 – 0.645 (**Table 6**). Sample A honey also had the best calibration R^2 for apparent sucrose (0.863 – 0.875), while sample C had the lowest R^2 (0.586 – 0.726) (**Table 7**). When spectral data of all honey samples were combined, the R^2 value was less than 0.78 and 0.87 for calibration and validation sets, respectively (data not shown). Further sampling may be needed to strengthen the robustness of calibration models for predicting sugar levels.

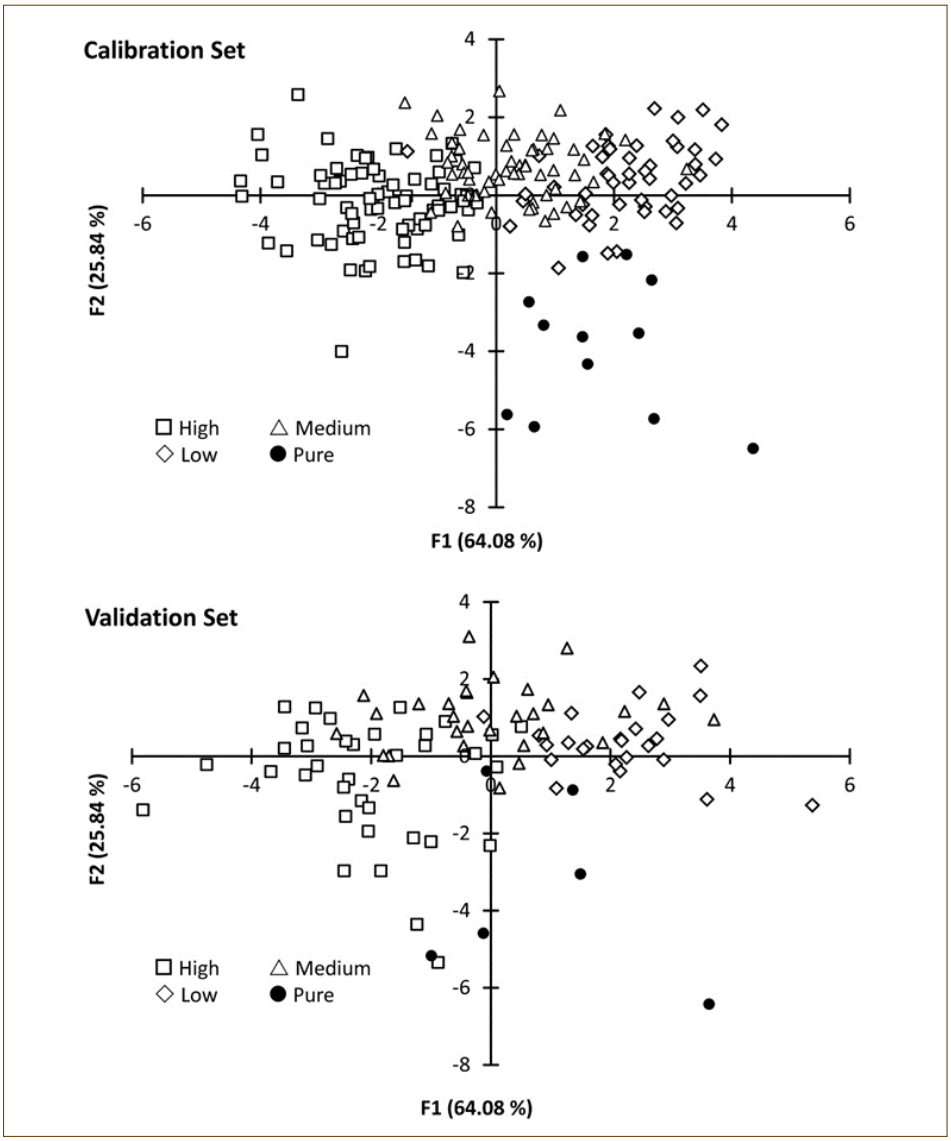

Figure 2. Scatterplot of the first two principal components of honey samples at different levels of adulteration using LDA for calibration (top) and validation (bottom) sets. Figures in percent correspond to the contribution of each principal component to the prediction.

Principal Component Analysis and Linear Discriminant Analysis

For spectra pre-treated with MSC and subjected to PCA, the eigenvector values of the first four PCs showed similar grouping of scores at 938 – 940 nm corresponding to water and two PCs for sugar at 838 – 840 nm. Similarly, for spectra subjected to SG – 1D – MC, eigenvector values showed similar grouping of scores at 978-980 nm (4 PCs), 984-986 nm (2 PCs) and 992-994 nm (2 PCs) corresponding to water; groupings at 836-838 nm (4 PCs) and 978-980 nm 314 (4 PCs) were also observed that correspond to sugar. These identified wavelengths are consistent with previous research of Williams and Norris (1987) (**Table 8**). Carbohydrates include saccharides and polysaccharides (e.g. sugars and starches) and cellulose (e.g. lignin-type bio-

Table 8. Absorption bands of selected molecules in the NIR wavelength range (nm)[z]

Water	Sugar	Starch	Cellulose
834	838	878	860
938	888	901	905
958	913	918	920
978	978	979	978
986	1005	1030	1058
994		1053	
1010			
1030			
1099			

[z]*Source: Williams and Norris (1987)*

Table 10. Classification accuracy (%) of linear discriminant analysis of near-infrared spectra of honey samples according to level of apparent sucrose[z]

Actual Level of Apparent Sucrose[y]	Predicted Level of Apparent Sucrose		Total Samples
	Authentic Honey	Adulterated Honey	
CALIBRATION SET			
Authentic Honey	100	0	26
Adulterated Honey	0	100	166
VALIDATION SET			
Authentic Honey	0	100	11
Adulterated Honey	0	100	85

[z]*Highlighted values indicate percentage of correctly classified samples*
[y]*Authentic Honey < 6% apparent sucrose; Adulterated Honey > 6% apparent sucrose*

Table 9. Classification accuracy (%) of linear discriminant analysis of near-infrared spectra of honey samples according to level of adulteration[z]

Actual Adulteration Level[y]	Predicted Adulteration Level				Total Samples
	None	Low	Medium	High	
CALIBRATION SET					
None	100.00	0.00	0.00	0.00	12
Low	0.00	100.00	0.00	0.00	48
Medium	0.00	1.67	98.33	0.00	60
High	0.00	0.00	0.00	100.00	72
					192
VALIDATION SET					
None	0.00	0.00	0.00	100.00	6
Low	0.00	75.00	20.83	4.17	24
Medium	0.00	0.00	90.00	10.00	30
High	0.00	0.00	8.33	91.67	36

[z]*Highlighted values indicate percentage of correctly classified samples*
[y]*None = pure honey, Low = 1-9%, Medium = 10-19%, High = 20-30%*

molecules) that consist mostly of aliphatic cyclic groups with attached OH groups and either linkage. Hence, the functional group at 836 nm is C-H methylene C-H, associated with branched aliphatic RC $(CH_3)_3$ or $RCH(CH_3)_2$ with its material type (hydrocarbons, aliphatic). At 979 nm, the functional group is O-H from water at near 0°C (Workman and Weyer, 2012).

LDA showed that 99.5% and 81.3% of all honey samples of the calibration and validation sets, respectively, could be correctly classified (**Table 9**) based on level of adulteration. The best results were obtained for samples with high levels of adulteration. Fourteen principal components were needed to classify honey according to adulteration level; a plot of the first two PCs is shown in **Figure 2**. In terms of apparent sucrose, 100% and 88.5% of all calibration and validation samples were correctly classified by LDA, respectively (**Table 10**); only one principal component was needed for separation (**Figure 3**). It should be noted, however, that all the authentic honey samples in the validation set were misclassified as adulterated. The results highlight the need for a larger set of pure samples to incorporate into the calibration set to improve classification.

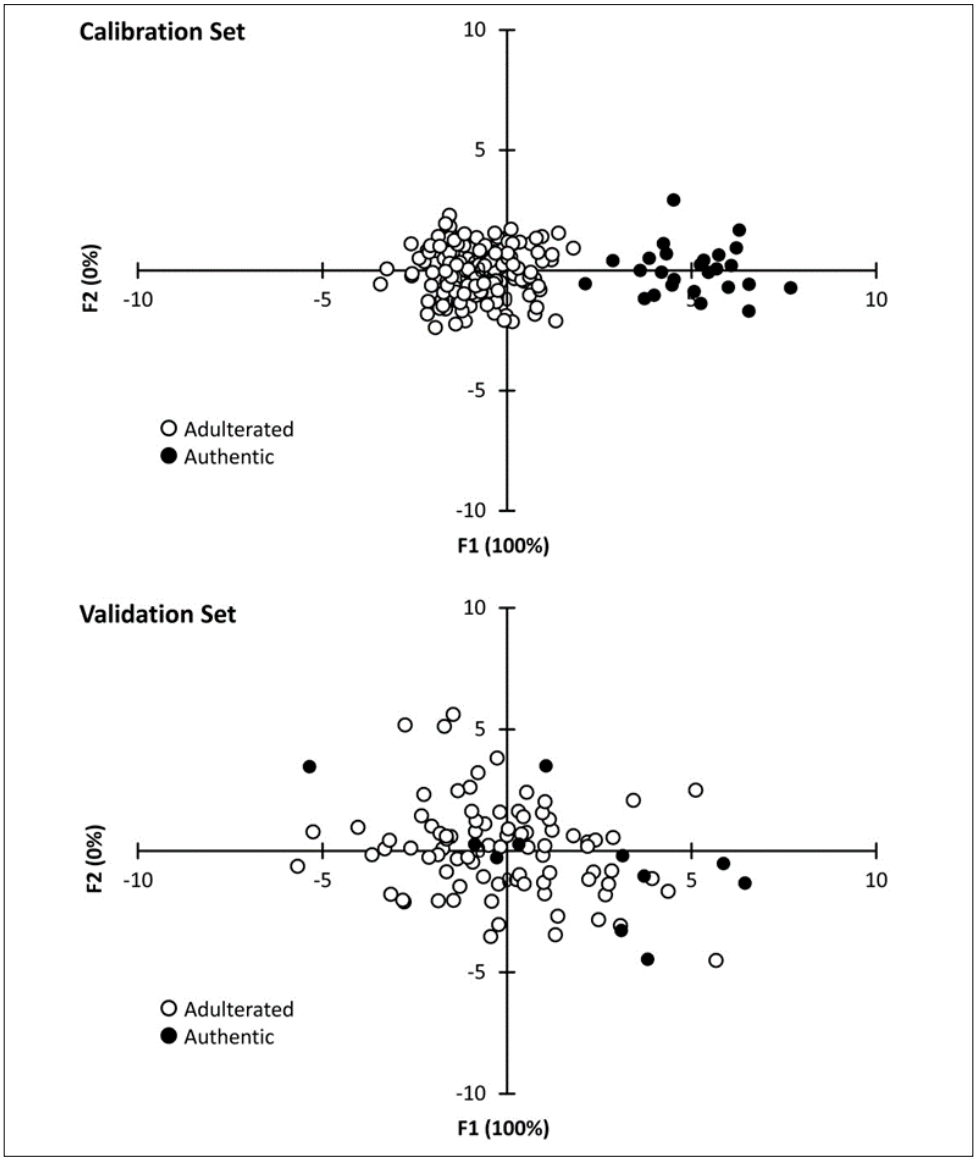

Figure 3. Separation of authentic and adulterated honey based on apparent sucrose content using linear discriminant analysis on calibration (top) and validation (bottom) sets. Figures in percent correspond to the contribution of each principal component to the prediction.

SUMMARY AND CONCLUSIONS

The present study showed that near infrared spectroscopy could potentially be used for predicting adulteration levels in Philippine honey. Honey sourced from six (6) different farms was adulterated with different amounts of prepared sucrose solutions and scanned with short-wave near-infrared light. Analysis of pre-processed spectra (700-1000 nm) showed that level of adulteration, level of reducing sugars, and total sugars could be predicted using partial least squares regression. Significant wavelengths were identified using principal component analysis and linear discriminant analysis; previous studies have associated these wavelengths with chemical bonds present in sugar and water. The best calibration and validation results were obtained using PLSR to

predict adulteration level (from pure honey to highly adulterated honey), indicating the potential of NIRS to rapidly test samples.

RECOMMENDATIONS

Further studies are needed to strengthen calibration models through more extensive sampling with respect to honey sources, production areas, and sampling times. This will help build up a spectral library of pure honey types that can be used for identifying and authenticating these highly-valued food products, and rapidly determining compliance with national and international product standards. The world standard for honey (CODEX STAN 12-1981 Rev. 1 (1987)) defines and specifies different types of honey according to source and method of extraction; limits on moisture content, sugar levels, and insoluble solids content are also described (Krell 1996). The Philippine Bureau of Agriculture & Fishery Standards (BAFS) has recently developed a product standard for honey (PNS/BAFS 185:2022), with specified limits on moisture content, sugar content (sucrose, fructose, and glucose), insoluble solids content, and carbon-stable isotope ratio. The use of NIRS in the honey industry should be increased to support these standards, ensure that products can be rapidly tested, and consumers protected from food fraud.

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