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Using FTIR-ATR and Chemometric Methods to Detect Sucrose Adulteration in Commercial Honey Samples

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ABSTRACT

Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR–ATR) was used to analyze pure and adulterated honey samples. The FTIR spectra was analyzed using principal component analysis (PCA) and partial least squares (PLS) regression analysis to determine if these methods could differentiate between pure, commercial, and sucrose-adulterated honey samples. PCA showed a clear distinction between pure and adulterated honey samples. Commercial honey samples showed clustering around the unadulterated samples. PLS regression analysis correctly identified 81.8% of the standards and samples used in the PCA analysis. The five commercial samples were tested and shown to have a concentration of less than 3% adulterant, which is likely due to differences in sucrose concentration between batches from different locations and bee types. PCA and PLS methods provide a quick and easy analysis of honey samples.

INTRODUCTION

Honey is a popular food item across the world and is widely consumed by humans. The honeybee, *Apis mellifera,* produces honey by consuming nectar from plants such as flowers and trees and converting it into honey using special enzymes that are contained within glands of the bee. The chemical composition of nectar varies between each plant. While the chemical composition may vary between different plants, the main components are sugars such as D-fructose, D-glucose, and sucrose. Other chemical constituents include amino acids, alpha-dicarbonyls, aromatic compounds, flavors, antioxidants, antibacterials, and minerals (Ball, 2007; Marceau, 2009). Location plays a critical role in the chemical composition of honey due to the differences in the plants that are available to the bees (Rios-Corripio, 2011). The enzymes that are used in the production of honey convert sucrose to D-fructose and D-glucose which are stored along with water and the rest of the components such as amino acids or aromatic compounds from

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the nectar. With the demand of honey being so high across the world, there are companies and producers of honey that seek to profit off the sale and distribution of honey. Some producers will add chemicals such as flavor compounds or other sugars to their products to produce a blend of honey that will fare well in the market. This adulteration of honey is harmful to the local producers of honey who do not have as large of a clientele as commercial producers. The adulteration of naturally produced honey could potentially lead to health risks as well if the product is adulterated with impure sugars, flavor compounds, or antioxidants (Cengiz, 2019; Kelly, 2004; Sahlan, 2019). These adulterated products are not tested or deemed safe by the FDA and could lead to harm in consumers if the adulterers added unsafe chemicals to their products. The need to qualify and quantify this information is important for the safety of consumers and for the economic safety of local producers who only produce and sell natural, unadulterated honey.

Many different techniques have been used to detect the adulteration of natural honey including NMR, HPLC, carbon-isotope ratio analysis, gas chromatography, and rheological methods (Cengiz, 2019; Kelly, 2004; Sahlan, 2019). While these methods are useful and effective, they take time and sample preparation. FTIR-ATR methods in the MID-IR allow for a faster and cheaper method of analyzing honey for adulteration due to the ease of operation, speed, cost, and the ability to analyze the method non-destructively. Different vibrational and rotational translations of certain functional groups allow for the detection of different chemicals within the honey mixture (Cengiz, 2019; Kelly, 2004; Sahlan, 2019). The spectral region between 882-944 cm^{-1} can be used to examine the concentration of sucrose in honey. Since the FTIR-ATR spectrum of honey varies between every sample, differences in the sucrose peaks between samples is hard to detect with the naked eye.

The use of chemometrics and multivariate analysis is necessary to distinguish between each category of sample. Principal Component Analysis is a multivariate method that is useful when analyzing large data sets and is used in the forensics and food sciences industries (Kamil, 2015). PCA decreases the dimensionality of a data set by creating new variables, called principal components, through linear combinations of the original data. These new points can be plotted on a 2- or 3-dimensional plot depending on how many principal components are used for the data analysis. Two principal components were used for this analysis. The points are arranged through space according to how correlated or uncorrelated they are to one another.

MATERIALS AND METHODS

Pure honey and adulterated honey were analyzed using a Nicolet IS5 iD7 FTIR equipped with a zinc-selenide ATR and analyzed by Origin-Pro data analysis application. A concentrated sucrose solution was made by dissolving 212 grams of granular sucrose in 100.13 grams of deionized water. This was used as the adulterant and added to pure samples of honey to produce adulterated samples that were between 6% and 22% sucrose solution by weight. Commercial honey products were purchased from the local grocery store and stored in the lab at room temperature.

The spectral region from 882-944 cm^{-1} was analyzed and used for the construction of a Partial-Least Squares regression model and a Principal Component analysis model to determine if

each adulterated sample could be distinguished from the pure samples of honey. Food Lion brand honey was used for the PLS regression model ranging from 0% to 27.22% sucrose by weight. The PCA model was used to distinguish between groups: unadulterated honey, adulterated honey at 7% sucrose, adulterated honey at 14% sucrose, adulterated honey at 21% sucrose, and each commercial honey brand. A total of 48 spectral files were used for the PCA model and a total of 10 samples were used to produce the calibration curve for the PLS model. Origin-Pro PLS and PCA analysis applications were used to predict the level of adulteration of each commercial honey sample as well as the prepared samples.

Five commercial honey brands were analyzed in triplicate to determine if there were any levels of sucrose adulteration in the samples. The five brands were: White Forest Honey, Goya orange blossom honey, Manischewitz clover honey, Golden Farms honey, and Nature's Promise honey. Each sample was purchased from the local Food Lion.

RESULTS

Principal Component Analysis

The PCA plot shows each group of honey separated out in nice thin bands. The four different levels of adulteration are clearly separated. 100% of the variability in the data is accounted for using the first two principal components. A 95% confidence interval is included for each group as well. The score plot for the principal component analysis is shown in Figure 1 below.

Partial Least Squares Regression

A PLS regression model was produced using the Origin-Pro data analysis software and Microsoft Excel. The predicted vs actual adulteration percentage plot shown in Figure 2 below has an equation of $y = 0.9988x + 0.0161$ with an R^2 value of 0.9988. The PLS model was used to predict the concentration of each sample that was used in the PCA model as well as the concentration of each commercial honey sample. The calculated concentration of each sample is listed in Table 1.

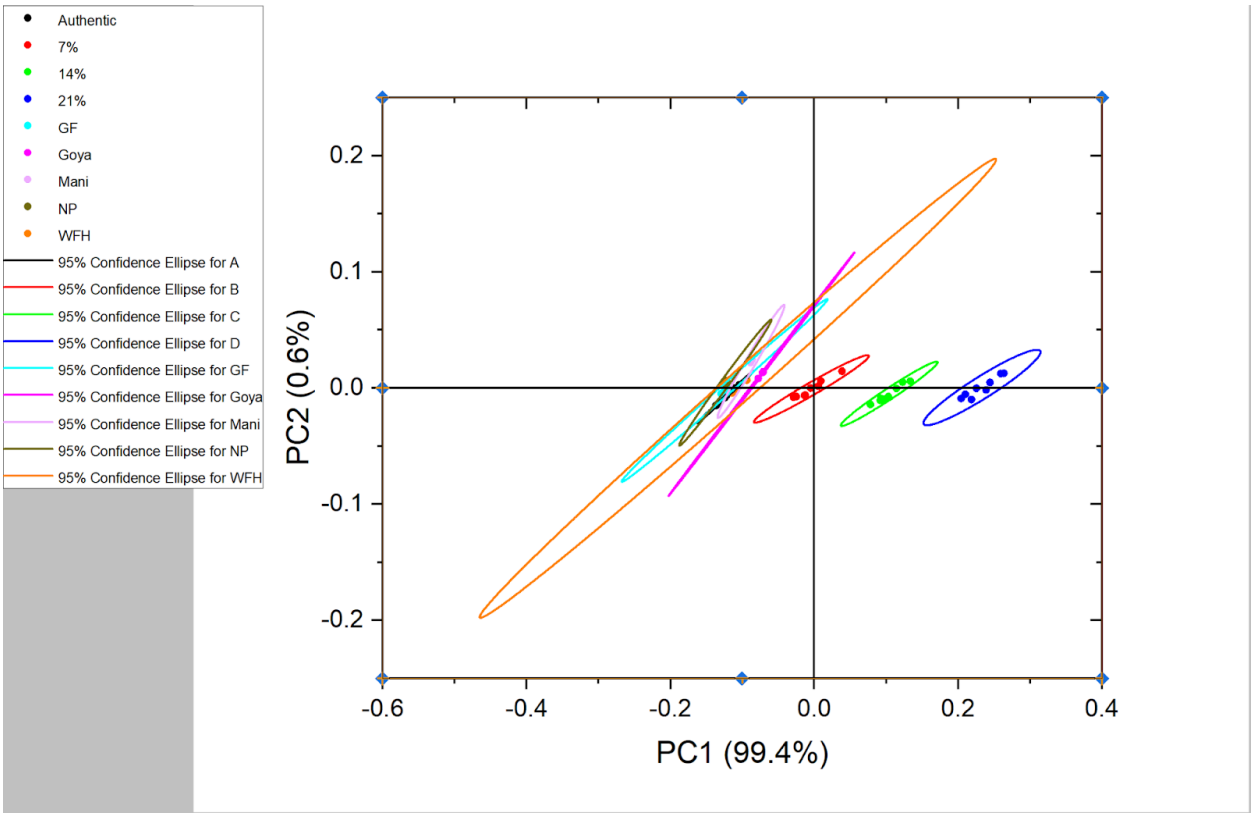

Figure 1: Score plot of each standard honey solution and commercial samples

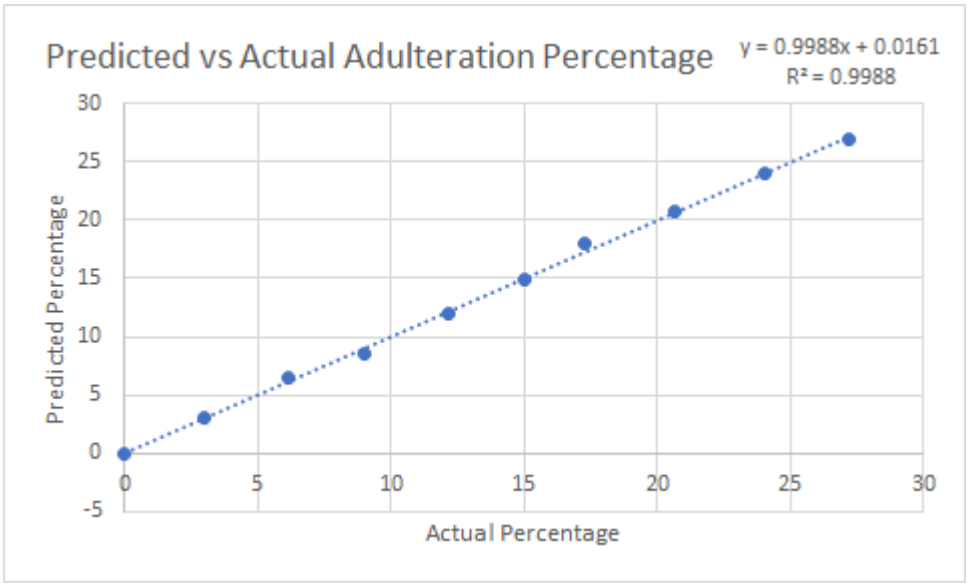

Figure 2: PLS regression model

Table 1: Percentage sucrose adulteration and predicted sucrose adulteration in 33 honey standards

Sample ID	Actual Percent Sucrose Solution	Predicted Percent Sucrose Solution	Percent Difference
A1	0	0.85125	200
A2	0	1.21415	200
A3	0	1.4338	200
A4	0	2.78683	200
A5	0	2.21548	200
A6	0	2.84408	200
A7	0	2.69805	200
A8	0	2.4948	200
A9	0	1.90486	200
B1	6.9	7.43752	7.498089
B2	6.8	7.19152	5.596533
B3	6.5	7.55044	14.95241
B4	7.1	7.94052	11.17674
B5	7.1	8.02862	12.27633
B6	7.2	6.72039	6.890755
B7	6.7	6.78267	1.226315
B8	6.9	7.35974	6.448084
C1	14	14.39514	2.783152
C2	13.8	13.47909	2.352791
C3	14.2	14.10036	0.704161
C4	14	15.03933	7.158085
C5	13.9	14.90923	7.006296
C6	14	14.92098	6.36894
C7	13.8	14.64461	5.938629
C8	14.2	14.87875	4.668357
D1	21.7	22.08961	1.779463
D2	20.9	21.48951	2.781396
D3	21.7	22.53584	3.779017
D4	20.7	20.94383	1.171026
D5	20.8	20.84283	0.205702
D6	20.9	20.29573	2.933654
D7	21.2	20.40079	3.842283
D8	21.1	20.72325	1.801629

DISCUSSION

Principal Component Analysis

The results of the principal component analysis show that as more concentrated sucrose solution is added to the honey, the further the points lie from the original non adulterated honey points. The black points in Figure 1 show the honey samples that contain no adulterant. These points are hard to see because of the overlap with the commercial products, but they cluster approximately -0.1 units to the left of the origin. The adulterated samples move to the right of the origin as the total solution increases the content of concentrated sucrose solution. The points that lie furthest to the right are the honey samples that are approximately 21% sucrose solution. The commercial samples cluster around the pure honey samples which indicate that there is no adulteration with sucrose solution or sucrose syrups. Since the data that was acquired had very little differentiation between each sample, almost 100% of the variability between each sample is described with the first two principal components. This removed the necessity for data pre-treatment to be done before analyzing the data.

Partial Least Squares Regression

Ten data points were used for the construction of the PLS regression model, starting with pure honey and gradually increasing the percentage of sucrose solution until a maximum of 27.22% was reached. The PLS model was used to predict the level of sucrose adulteration in each of the samples used in the PCA model as well as the commercial samples. Samples with predicted values that differ from the actual values by greater than 8% are considered misclassified. Only three samples were misclassified by using this method. The three misclassified samples were in the 7% adulterated category. The rest of the samples were correctly classified into their categories using the PLS model. Using the formula for percent difference shown in Equation 1, the percent difference for each authentic sample is 200%. Any calculated concentration over 2.5% sucrose solution is treated as a misclassification. Three standards were calculated to have a concentration of greater than 2.5%. Using the PLS regression model, 81.8% of the total standards were correctly classified. This is similar that was has been observed for other countries where water and vinegar additions were done. (Mail, 2019; Riswahyuli, 2020)

The commercial samples were analyzed in triplicate and an average concentration of added sucrose solution was calculated using the regression model. The results of these are shown in Table 2. Based on the PLS model and the PCA model, the commercial samples all had predicted concentrations of sucrose solution below 3% while also clustering around the unadulterated points. The highest average concentration of the commercial products was White Forest Organic honey at 2.8%, while the lowest average concentration was Manischewitz clover honey at -2.0%. Based on the PLS model and PCA model, it does not appear that any of the commercial products were adulterated with sucrose. Differences in sucrose content between different brands of honey are due to different types of plants in the region in which the bees collected nectar. Different breeds of honeybee also produce different levels of sucrose in their honey based on the types and levels of enzymes present in each hive of bees. (Marceau, 2009)

Equation 1: Percent difference equation

$$\frac{|y - x|}{\frac{y + x}{2}}$$

Where y is the calculated value and x is the true value

Table 2: Predicted and average predicted concentrations of commercial products

Sample ID	Predicted Concentration	Average Predicted Concentration
GF-1	0.35358	0.658377
GF-2	0.98256	
GF-3	0.63899	
Goya-1	0.51104	0.746893
Goya-2	1.33861	
Goya-3	0.39103	
Mani-1	-1.92198	-2.02729
Mani-2	-1.85725	
Mani-3	-2.30265	
NP-1	-0.7989	-0.47912
NP-2	-0.1187	
NP-3	-0.51977	
WFH-1	2.20786	2.802403
WFH-2	2.43406	
WFH-3	3.76529	

GF = Golden Farms, Goya = Goya, Mani = Manischewitz,
NP = Natures Promise, WFH = White Forest Honey

CONCLUSION

FTIR-ATR was used to analyze pure and adulterated honey samples. We were able to differentiate between pure, commercial and sucrose adulterated honey samples by FTIR spectra analyzed by principal component analysis (PCA) and partial least squares (PLS) regression analysis. PCA showed a clear distinction between pure and adulterated honey samples. Commercial honey samples showed clustering around the unadulterated samples. PLS regression analysis correctly identified 81.8% of the standards and samples used in the PCA analysis. Five commercial samples were tested and shown to have a concentration of less than 3% of adulterant, which is likely due to differences in sucrose concentration between batches from different locations and bee types.

LITERATURE CITED

Ball, D. W. (2007). The chemical composition of honey. *Journal of Chemical Education, 84*(10), 1643-1646. https://doi.org/10.1021/ed084p1643

Cengiz, M. F. and Durak, M. Z. (2019). Rapid detection of sucrose adulteration in honey using Fourier transform infrared spectroscopy. *Spectroscopy Letters 52*(5), 267–273. https://doi.org/10.1080/00387010.2019.1615957

Kamil, M., Asri, M. N. M., Desa, W. N. S. M., and Ismail, D. (2015). Fourier transform infrared (FTIR) spectroscopy and principal component analysis (PCA) of unbranded black ballpoint pen inks. *Malaysian Journal of Forensic Sciences*, *6*(1), 48–53.

Kelly, J. F. D., Downey, G., and Fouratier, V. (2004). Initial study of honey adulteration by sugar solutions using midinfrared (MIR) spectroscopy and chemometrics. *Journal of Agricultural and Food Chemistry, 52*(1), 33–39. https://doi.org/10.1021/jf034985q

Mail, M. H., Rahim, N. A., Amanah, A., Khawory, M. H., Shahudin, M. A., and Seeni, A. (2019). FTIR and elementary analysis of *Trigona* honey, *Apis* honey and adulterated honey mixtures. *Biomedical and Pharmacology Journal, 12*(4), 2011-2017. https://doi.org/10.13005/bpj/1833

Marceau, E. and Yaylayan, V. A. (2009). Profiling of α-dicarbonyl content of commercial honeys from different botanical origins: Identification of 3,4-dideoxyglucoson-3-ene (3,4-DGE) and related compounds. *Journal of Agricultural and Food Chemistry*, *57*(22), 10837–10844. https://doi.org/10.1021/jf903341t

Rios-Corripio, M. A., Rios-Leal, E., Rojas-Lopez, M., and Delgado-Macuil, R. (2011). FTIR characterization of Mexican honey and its adulteration with sugar syrups by using chemometric methods. *Journal of Physics: Conference Series, 274*, 1-5, Article number 012098. https://doi.org/10.1088/1742-6596/274/1/012098

Riswahyuli, Y., Rohman, A., Setyabudi, F. M. C. S., and Raharjo, S. (2020). Indonesian wild honey authenticity analysis using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy combined with multivariate statistical techniques. *Heliyon*, *6*(4), Article number e03662. https://doi.org/10.1016/j.heliyon.2020.e03662

Sahlan, M., Karwita, S., Gozan, M., Hermansyah, H., Yohda, M., Yoo, Y. J., and Pratami, D. K. (2019). Identification and classification of honey's authenticity by attenuated total reflectance Fourier-transform infrared spectroscopy and chemometric method. *Veterinary World*, *12*(8), 1304–1310. https://doi.org/10.14202/vetworld.2019.1304-1310