FTIR characterization of Mexican honey and its adulteration with sugar syrups by using chemometric methods

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Abstract. A chemometric analysis of adulteration of Mexican honey by sugar syrups such as corn syrup and cane sugar syrup was realized. Fourier transform infrared spectroscopy (FTIR) was used to measure the absorption of a group of bee honey samples from central region of Mexico. Principal component analysis (PCA) was used to process FTIR spectra to determine the adulteration of bee honey. In addition to that, the content of individual sugars from honey samples: glucose, fructose, sucrose and monosaccharides was determined by using PLS-FTIR analysis validated by HPLC measurements. This analytical methodology which is based in infrared spectroscopy and chemometry can be an alternative technique to characterize and also to determine the purity and authenticity of nutritional products as bee honey and other natural products.

1. Introduction
Honey is a natural product of high nutritional value which is believed to be a target for adulteration. Sugar and water represent the main chemical constituents of honey (typically 80% carbohydrate and 17% water), whereas proteins, flavors and aromas, pigments, vitamins, free amino acids, and numerous volatile compounds constitute the minor components [1]. Given its composition, this would most simply take the form of extension by cheaper, commercially-available sugar syrups. Fourier transform infrared spectroscopy offers a fast and non-destructive alternative to chemical measurement techniques for qualitative characterization [2]. The multivariate methods such as principal component regression (PCR) [3] and partial least squares (PLS) [4] provide a useful way to develop prediction models able to predict several variables such as sugar contents or adulteration contents in honey or other food products. The first reports on this subject include those that showed the ability of near (NIR) and middle (MIR) infrared spectroscopy to detect adulteration of authentic honey by either added corn syrup (CS) [5], beet invert syrup (BI) [6], sucrose syrup (SS) [7], and more recently on adulteration of honey with high fructose corn syrup (HFCS) [8]. In addition to that, other researchers have reported on the chemometrical methods to provide a useful way to develop prediction models able to predict several variables such as sugar contents or adulteration contents in honey or other food products [9, 10]. In this work we have used a combination of FTIR spectroscopy and chemometry for
detection of adulteration of bee honey with two types of cheap syrups, such as corn syrup and cane sugar syrup. The bee honey samples studied in this research were recollected mainly from the central region of Mexico.

2. Experimental
Honey samples were gathered from artisanal beekeepers mainly from Tlaxcala and Puebla regions of Mexico. Samples were not independently tested for authenticity as each sample was collected from apiarists. All the samples were warmed up to 35 °C to dissolve solids and obtain an adequate viscosity, followed by a vigorous stirring. Once the samples reached the room temperature, their infrared absorption was measured, using only 20 μl. In addition to that, one sample of pure honey was intentionally adulterated with two cheap syrups: corn syrup (CS) and cane sugar syrup (CSS). Such adulterations were made at proportions of 10, 20, 30, 40, 50, 60, 70, 80 and 90% (adjusting previously both syrups and pure honey samples at 70° Brix). The honey proportion was 100% – X%, where X is the percentage of the adulterant solution.

Infrared spectra were obtained by using a Fourier transform infrared (FTIR) spectrometer, Bruker Vertex 70 equipped with an attenuated total reflectance (ATR) accessory. Spectral measurements were recorded in the wavenumber range between 650–4000 cm⁻¹ (MIR). Each spectrum was collected and ratioed against the background spectrum of the clean crystal surface in order to present the spectra in absorbance. Only two replicas of each sample were recorded because of the signal to noise ratio is very high. For example, the highest intensities of absorption are between 0.4 and 0.5, whereas the average intensity from noise is 0.01, giving a signal to noise ratio between 40 and 50.

3. Results and discussion
Figure 1 shows the FTIR spectra of honey samples in the region of sugars 900–1140 cm⁻¹. Such spectra show a group with a common line shape, which is attributed to pure honey; and also it is noticeable other group with different spectral features, which is attributed to adulterated samples. The last group contains spectra with absorptions enhanced in glucose and sucrose, and very different to the typical intensities expected for pure honey. These results suggest that some samples were adulterated with corn syrup (high content of glucose) CS, and with cane sugar syrup (high content of sucrose) CSS as shown in Figure 1. To make these results more clear, the principal component analysis method (PCA) was applied to whole group of FTIR spectra, obtaining an evident discrimination (score plot) of the pure honey with respect to the adulterated honey, as shown in Figure 2. It can be observed a clear splitting of the data associated to pure honey with respect to the adulterated honey, as depicted by the first two principal components in the sugars region. Figure 3 shows the score plot for the first three principal components; it can be observed with more detail the spatial regions described for the adulterations of honey according to the type of syrup used. This methodology represents a forceful test to evaluate the authenticity and also the type of adulteration in honey. After the Eigen-analysis of the covariance matrix (PCA), which was done by using Minitab software, the obtained contributions for the first three principal components are: PC1 (0.716), PC2 (0.150) and PC3 (0.123), giving an accumulative value of 0.866 for two components (Fig. 2), and 0.990 for three components (Fig. 3).

To observe the spectral changes due to adulteration, one sample of pure honey was intentionally adulterated with two cheap syrups: corn syrup (CS) and cane sugar syrup (CSS), as described in experimental section. Figure 4 shows the FTIR spectra of the adulteration of honey with several proportions of cane sugar syrup, this process starts from the pure honey to cane sugar syrup.
Figure 1. FTIR spectra of pure honey and also of adulterated honey in the region of the sugars.

Figure 2. Score plot (2D) obtained from the principal component analysis (PCA) applied to the FTIR spectra.

Figure 3. Score plot (3D) obtained from the principal component analysis (PCA) applied to the FTIR spectra.

Figure 4. FTIR spectra of the adulteration of honey with several proportions of cane sugar syrup.

In addition to that, partial least squares (PLS) analysis was applied to the FTIR spectra of pure honey, in order to know the concentration of some of the main sugars: glucose, fructose, sucrose and monosaccharides, as shown in figures 5-8. In this case high performance liquid chromatography (HPLC) was used as reference technique to validate the FTIR predictions of the concentration of sugars analyzed in this group of pure honey samples. These results show that the chemometric analysis coupled to FTIR spectroscopy can be used in a practical form to predict the content of sugars. PLS analysis implies the optimization of the parameters: $R^2$ (correlation coefficient), SEC (standard error of calibration) and SEP (standard error of prediction). These parameters were optimized by using QUANT software, and are shown in Figs 5-8.
Figure 5. Partial least squares (PLS) calibration curve that predict the glucose content in honey.

Figure 6. Partial least squares (PLS) calibration curve that predict the fructose content in honey.

Figure 7. Partial least squares (PLS) calibration curve that predict the sucrose content in honey.

Figure 8. Partial least squares (PLS) calibration curve that predict the monosaccharides content in honey.

4. Conclusions
It has been performed a chemometric analysis to characterize and determine adulteration in honey with cheap syrups which are used commonly for this practice. In first instance the FTIR spectra seem to have a clear form to determine the purity or adulteration of the honey samples, however, coupling of this technique with chemometry, as the principal component analysis (PCA) and partial least squares (PLS) methods, the results are more conclusive, and the discrimination of honey can be performed more easily. In addition to that, the use of PLS procedure, it was possible to quantify the concentration of the main sugars present in honey: glucose, fructose, sucrose and monosaccharides. This methodology can be applied in the analysis and characterization of honey and other food products in a practical way.
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