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Classification of honey using fourier transform infrared spectroscopy and chemometrics

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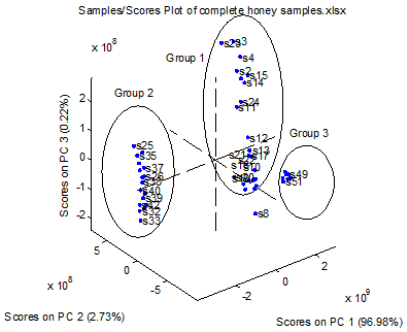
GRAPHICAL ABSTRACT



ABSTRACT

The different type of honey depend on the nectar collected from the flower by the bees and regional climatic condition and gave different composition of carbohydrates to one another. In this study the different types of honey were discriminated based on the differences in their molecular content and some of honey samples were adulterated to differentiate them from the natural one precisely. The following respective weight ratios: 0.7:1.0. 1.2:1.0 (typical of honey composition), and 2.3:1.0 of solutions containing both D-fructose and D-glucose were prepared for adulterant solutions and then was added to individual honeys at levels of 7, 14, and 21% w/w. The Fourier Transform Infrared Spectroscopy (FTIR) and attenuated total reflection (ATR) sampling have been used to detect the composition of all samples from 650 to 4000 cm^{-1} . For each spectra the range of 1000 to 1800 cm^{-1} were used as variable with 5cm^{-1} interval value of absorbance. The Chemometric method was used for all the samples by performing pattern recognition procedures and Principal Component analysis (PCA). The FTIR-ATR show to be good methodology to quantify the molecular content of sugar in honey and the Chemometric method is the pactical technique to differentiate the adulterant sample from the natural one.

Keywords: *Honey, Fourier Transform Infrared Spectroscopy (FTIR) analysis, MATLAB, Principal Component Analysis (PCA)*

1. INTRODUCTION

Honey, a natural product of very high nutritive value is made when the nectar (floral) and sweet deposits from plants (non-floral) are gathered, modified and stored in the honeycombs by honeybees of the genera *Apis* and *Meliponini*. Honey characteristics depend primarily on the botanical origin of nectar. Floral source of the nectar predominantly affects the chemical composition of honey in terms of its protein, carbohydrate, enzyme, mineral and organic acid content [1]. Nowadays, demand on honey is gradually receiving attention as a complementary and or an alternative source of treatment in modern medicines. It is active against antibiotic-sensitive and antibiotic-resistant strains of micro-organisms and has the potential not to select for further resistant strains. Honey Tualang one of the most expensive honey in the Malaysia due to its significant contribution to human health. Honey Tualang one the types of honey that was harvested from *Apis dorsata* bees' nectar on the Tualang tree. Honey Tualang is dark brown in an appearance compared to normal honey which has more light color. It has been used traditionally for the treatment of various diseases, where its therapeutic value has partly been related to its antioxidant properties. Honey is highly prized by consumers as natural sweet substance. Honey is essentially concentrated aqueous solution of inverted sugars, but it also contains a very complex mixture of other saccharides, proteins, enzymes, amino acids, organic acids, polyphenols, and carotenoid like substances, maillard reaction products, vitamins and minerals.

Carbohydrates constitute about 95 to 97% of the dry weight of honey. Fructose and glucose are the most predominant sugars present and are responsible for most of the physical and nutritional characteristics of honey.

2. EXPERIMENTAL

In this project, 24 samples of honey purchased from the local supermarket will be analyzed using FTIR. The grouping formed in pattern recognition analysis for different types of honey samples will be analyzed. The digital spectra of adulteration samples and authentic sample of honey will be used in pattern recognition methods to differentiate the adulterated samples from pure honey samples.

2.1. Honey samples

In this study, 24 samples of honey that come from various types and origin are purchased from the market throughout Malaysia.

2.2. Sample Preparation

All the samples were stored in air-tight jars at 4°C. Prior to spectroscopic analysis, honey samples were incubated in a warm water bath to dissolve solids and obtain an adequate viscosity. Aqueous solution were prepared using distilled water and containing fructose and glucose in the following (F: G) ratios by weight 0.7:1.0, 1.2:1.0, and 2.3:1.0. Each adulterant sugar solutions and all the honey samples were diluted with distilled water to 30° Brix which is measured by using refractometer. Three authentic honey samples were adulterated at level 7, 14, and 21% w/w using each of the adulterant solutions. Prior to FTIR spectroscopy analysis, all samples were placed in an oven overnight at 40°C to dissolve any crystalline material present and stir manually to produce a homogeneous solution.

2.3. FTIR Analysis

Infrared spectra were obtained by using a Fourier transform infrared (FTIR) spectrometer, Bruker Vertex 30 equipped with an attenuated total reflectance (ATR) accessory. Spectral measurements were recorded in the wavenumber range between 650–4000 cm^{-1}[2].

2.4. Data Acquisition

From the FTIR analysis, the raw data of 51 honey samples were obtained. These data were saved in a file with *.SP (spectra) extension which is the plot of absorbance versus wavenumber. The spectra were used to perform pattern recognition.

2.5. Conversion of Raw Data into Digital Data

All the spectrum collected from FTIR analysis were converted into the digital variables in the data processing that used for performing the pattern recognition. The common Microsoft Word processing
programs such as Notepad and Microsoft Office known as JCAMP-DX with extension *DX were used in the conversion of *SP file into the format that digital data can be read.

2.6. Variable Selection

The data manipulation and chemometrics processing were done in MATLAB. However, it is a software works with matrices of data. Consequently, it is necessary to convert the digital data of spectra into a data matrix which is readable by MATLAB. The software Microsoft Excel (MS Excel) is used for the data conversion process. The data matrix was constructed to compare the honey classification model results. The data matrix was simply constructed by using selecting digital data method, where the honey absorbance was selected for every 5 cm^{-1} wavenumber in the spectrum as variables in the range of 1800 cm^{-1} to 1000 cm^{-1}.

2.7. Pattern Recognition

The data manipulation and chemometrics processing were done in MATLAB. However, it is a software works with matrices of data. Consequently, it is necessary to convert the digital data of spectra into a data matrix which is readable by MATLAB. The software Microsoft Excel (MS Excel) is used for the data conversion process. The data matrix was constructed to compare the honey classification model results. The data matrix was simply constructed by using selecting digital data method, where the honey absorbance was selected for every 5 cm^{-1} wavenumber in the spectrum as variables in the range of 1800 cm^{-1} to 1000 cm^{-1}.

2.8. Principal Component Analysis

The data manipulation and chemometrics processing were done in MATLAB. However, it is a software works with matrices of data. Consequently, it is necessary to convert the digital data of spectra into a data matrix which is readable by MATLAB. The software Microsoft Excel (MS Excel) is used for the data conversion process. The data matrix was constructed to compare the honey classification model results. The data matrix was simply constructed by using selecting digital data method, where the honey absorbance was selected for every 5 cm^{-1} wavenumber in the spectrum as variables in the range of 1800 cm^{-1} to 1000 cm^{-1}.

3. RESULTS AND DISCUSSION

The main discussion in this chapter will be the result FTIR-ATR analysis of honey sample. Then, followed by pattern recognition using Principal Component Analysis (PCA). The honey classification models (scores plot) in PCA were conducted in several steps such as selecting suitable number of principal components, identifying groupings using scores plot and identify variables use in loading plot.

3.1 FTIR Spectrum Analysis

In this study, FTIR-ATR were used to compare honey samples based on their spectral differences in the 4000-650 cm-1 spectral region. A representative of spectrum of honey as shown in figure 1.1. Table 1.1 represent the band assignment along with corresponding modes of vibrations in the FTIR-

ATR spectrum of pure honey sample from Selangor. Figure 2 shows comparative infrared spectra of all authentic honey samples in the region 4000-650 cm^{-1}. There are a total 24 authentic honey samples analyzed using FTIR Spectrometer and in the figure 2 the spectral differences between the group were clearly seen.

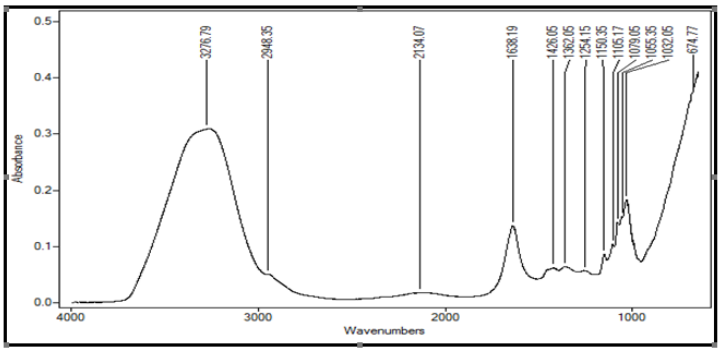


Figure 1.1 FTIR spectrum for Pure Honey

Table 1.1 Infrared Band Assignments for Pure Honey.

Frequency (cm^{-1})	Functional group and mode of vibration
3276.79	O-H (H-bonded)
2948.35	C-H stretching
1638.19	O-H (Water) bending
1426.05	$-CH_2$ bending (strong)
1362.05	$-CH_2$ bending (medium)
1254.15	C-C, O-CH, C-OH bending
1032.05	C-O stretching

For the adulterated honey samples, there were 27 spectra obtained from the FTIR-ATR analyst of 27 adulterated samples of honey (7% w/w, 14% w/w and 21%w/w) with different ratios of fructose to glucose (0.7, 1.2 and 2.3). The spectrum of authentic honey samples as shown in Figure 1.2 together with spectra of the three adulterant solution (14% w/w) are shown in Figure 1.3.The adulterated honey samples spectral show a shift to longer wavelength and a broadening of the absorption bands as the concentration of fructose increase. This FTIR-ATR analysis show that the adulteration of honey can also be detected by analyzed the spectrum shape.

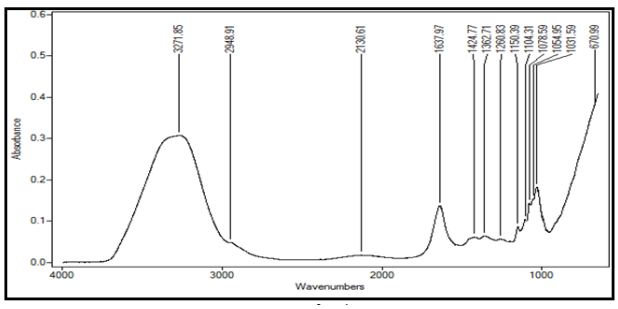


Figure 1.2 FTIR spectrum for Kira HAQ Pure Honey

3.2. Principal Component Analysis

The PCA model was develop from the data matrix of 24 authentic honey samples and 27 adulterant honey samples by importing into PCA tool. Before the classification model honey samples was constructed, the mean center mode have been selected as the preprocessing mode because all honey samples used the same unit which is the absorbance of the honeys. The differences of unit will use different preprocessing mode [3].

3.3. Score Plot Analysis

The score plot represent the relationship between honey samples as it related to each other with respect to the measurement variables. The score plot is demonstrated as the two-dimensional or three-dimensional graphs as it plotted with PCs as axes. The similar characteristic of each samples were determine by the group that will formed. The development of the two model in this study were carried out the variables selection method and it will used in order to get scores plot results for the method to be used in classifying the samples.

The original data set of honey samples was 51 x 161 matrix that used in this study. The two-dimensional plot with PC1 (96.98%) and PC2 (2.73%) as the axes as shown in Figure 1.3. As the grouping of the samples not clearly show in the two-dimensional plot, so the three-dimensional plot was constructed with PC1, PC2 and PC3 as axes as shown in Figure 1.4.

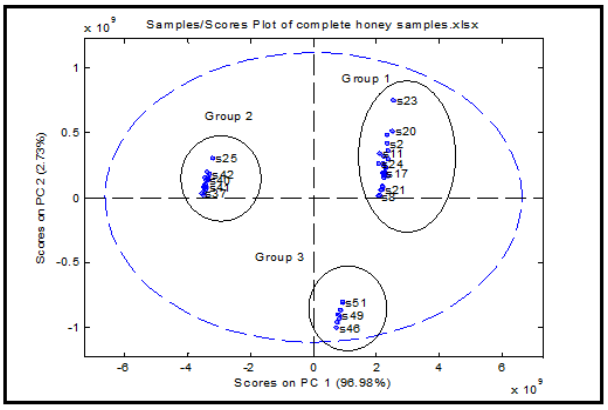


Figure 1.3 Scores plot of PC 2 versus PC 1

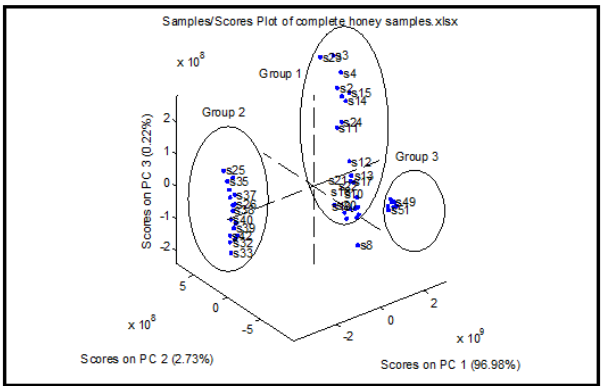


Figure 1.4 Scores plot of PC3 versus PC 2 versus PC 1

From the score plot PC1 versus PC2 can be seen that there were three groups of honey samples. The score plot were grouped as the pure honey and adulteration honey. The adulterated honey was added some other percentage of sugar which are 7%, 14% and 21% with different ratios of fructose to glucose. The group one are represent the authentic honey samples which were sample 1, 2, 3, 4, 5, 6,7,8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22,23 and 24.

Meanwhile, the other two group were the adulterant honey samples which were group two and group three. The score plot show that the purity of honey in the market generally can be trust as all the authentic honey samples were grouped in group one. The blue color dotted circle indicated that all the sample are in near area and located in the 95 percent confidence level.

The two-dimensional score plot cannot differentiate the different type of pure honey samples although they are from different origin as they all clustered near to each other in one group. So, the three-dimensional score plot that involve PC1, PC2 and PC3 were constructed as shown in figure 4.6. The three-dimensional score plot shows one of the authentic honey samples can be differentiated from others as it located far away from other samples in group one which was sample 8. This is probably because the third component detected the special characteristic that own by sample 8. However, the other sample's of Tualang honey which were sample 4, 6 and 15 cannot be detected by the third component as they were clustered close to other samples in group one. This show that origin of the samples cannot easily detected and distinguish by using only FTIR-ATR analysis and principal component analysis.

According to the theory, each PC represent certain factor of variation where PC 1 represent the general variance of factors such as origin, taste, and texture of the samples. This shows that the variance of factor that PC. The purity of honey samples is used to construct the class. The honey samples from the market are really pure honey based on the grouping that formed because it is not grouped in the adulterated honey. Table 4.2 shows the samples in which group.

Table 1.2: Honey Sample Number, Name and Group based on PCA

Samples Number	Honey brands/sources	% w/w	Fructose: Glucose	Group
1	Kira HAQ Honey	-	-	1
2	Nusantara Pure Honey	-	-	1
3	Barang Kita Honey	-	-	1
4	Tualang Pontian Honey	-	-	1
5	Angsana Pure Honey (1)	-	-	1
6	Tualang Special Batu Pahat	-	-	1
7	Pure Angsana Honey (2)	-	-	1
8	Tualang Kuala Terengganu	-	-	1
9	Giant Pure Honey	-	-	1
10	Pure Pekan Honey	-	-	1
11	Cameron Highland Honey	-	-	1
12	Pure Kurma Market Honey	-	-	1
13	Kelulut Skudai Honey	-	-	1
14	Pure Skudai honey	-	-	1
15	Tualang Chini Honey	-	-	1
16	Muhibah Pure Honey	-	-	1
17	Al-Shifa Honey	-	-	1
18	Herbal Honey	-	-	1
19	Organic Tesco Honey	-	-	1
20	Giant Bee Honey	-	-	1
21	Natural Fraser Hills Honey	-	-	1
22	Organic Herbal Honey	-	-	1
23	FAMA Pure Honey	-	-	1
24	Pure Ledang Honey	-	-	1
25	Kira HAQ Honey	7	(0.7:1.0)	2
26	Kira HAQ Honey	14	(0.7:1.0)	2
27	Kira HAQ Honey	21	(0.7:1.0)	2
28	Kira HAQ Honey	7	(1.2:1.0)	2
29	Kira HAQ Honey	14	(1.2:1.0)	2
30	Kira HAQ Honey	21	(1.2:1.0)	2
31	Kira HAQ Honey	7	(2.3:1.0)	2
32	Kira HAQ Honey	14	(2.3:1.0)	2
33	Kira HAQ Honey	21	(2.3:1.0)	2
34	Nusantara Pure Honey	7	(0.7:1.0)	2
35	Nusantara Pure Honey	14	(0.7:1.0)	2
36	Nusantara Pure Honey	21	(0.7:1.0)	2
37	Nusantara Pure Honey	7	(1.2:1.0)	2
38	Nusantara Pure Honey	14	(1.2:1.0)	2
39	Nusantara Pure Honey	21	(1.2:1.0)	2
40	Nusantara Pure Honey	7	(2.3:1.0)	2
41	Nusantara Pure Honey	14	(2.3:1.0)	2
42	Nusantara Pure Honey	21	(2.3:1.0)	2
43	Pure Kurma Market Honey	7	(0.7:1.0)	3
44	Pure Kurma Market Honey	14	(0.7:1.0)	3
45	Pure Kurma Market Honey	21	(0.7:1.0)	3
46	Pure Kurma Market Honey	7	(1.2:1.0)	3
47	Pure Kurma Market Honey	14	(1.2:1.0)	3
48	Pure Kurma Market Honey	21	(1.2:1.0)	3
49	Pure Kurma Market Honey	7	(2.3:1.0)	3
50	Pure Kurma Market Honey	14	(2.3:1.0)	3
51	Pure Kurma Market Honey	21	(2.3:1.0)	3

4. CONCLUSION

The chemometric method can be used in large number of data that can be extracted to get more information from the FTIR spectrum of samples. In this study, it shows that possible to tell apart FTIR spectrum of honey from those of honey samples adulterated with sugar solutions. The coupling this technique with chemometric analysis, as the principal component analysis (PCA) the result are more certain, and the discrimination of honey can be performed more easily [4]. Thus, this method more easily to identify the group and characterizing of the samples.

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