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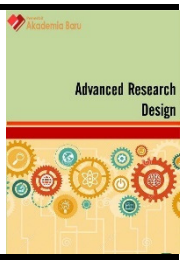

Classification of Malaysian honey using Fourier transform infrared spectroscopy and principal component analysis

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ABSTRACT

Honey is a natural sweetener, which is consumed in a variety of sweet products. It is considered as healthy food because it contains nutrients such as carbohydrate, protein, vitamins and mineral. The presence of adulterated honey in the market is worrying the consumers since it is difficult to distinguish between pure and adulterated honey due to similar appearance and texture of both type honeys. Chemometric analysis combined with spectroscopic data is a powerful technique that has been used to discriminate different type of honey. Samples of pure honey are collected from beekeepers at Ayer Keroh, Melaka and Cameron Highland, Pahang. The adulterants used to prepare adulterated honey are sugar and corn syrup with the concentration of the adulterants added to the pure honey ranging from 10% to 90% by weight of adulterant. All the samples are treated with heat at 40^{o}C to ensure the adulterant and pure honey are mixed well. Fourier transforms infrared spectroscopy (FTIR) is used to generate the spectra of the honey and subsequently subjected to chemometric analysis. The spectra data is then analysed by using Principal Component Analysis (PCA) technique using SOLO+Mia software. In this study, all honeys have been successfully discriminated according to their origins and purity as well as types of adulterants used. Consequently, the developed model can potentially be used as a screening tool to determine the purity of honey in the market.

1. Introduction

Honey is obtained from the nectars of flowers produced by honey bee (Apis mellifera). There are about 200 substances in honey and the major constituents are carbohydrate and water. Other chemical constituents of honey include acid, vitamins, protein, mineral and enzyme. However, the constituents of honey may change and undergo some chemical reaction such as oxidation,

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fermentation, decomposition, dehydration and thus change the quality of honey during the process of producing the honey such as packaging, processing and transportation of honey [1].

Nowadays, consumers are concern about the authenticity of the honey they consumed. The presence of adulterated honey in the market has affected the price and quality of the honey as well. It is believed that the adulterated honey may has some adverse effects on health as the consumer is not given prior information about the adulterants used in honey. In general, the common substance used in adulteration of honey is corn syrup, sugar, high fructose and maltose and also honeybees fed with sucrose [2]. Thus, it is critical to discriminate amongst the authentic and adulteration honey to ensure that the honey we consume is beneficial to our health [3].

Based on previous work, the authenticity of honey can be determined by using chromatographic technique such as High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC). Unfortunately, the techniques require high cost, high skilled operator and destructive to the sample [4]. The duration to analyse the sample by using these techniques are considered longer compared to the spectroscopic technique. Thus, Fourier Transform Infra-Red (FTIR) can be used as alternative method to analyse the quantitative measurement of sample by combining with the multivariate data analysis where principal component analysis (PCA) will be used as pattern recognition technique. Several works has been reported on the usage of FTIR to analyse food sample [5] and honey [6]. In this study, the combination of FTIR and PCA is employed to classify honey based on the purity as well as origin and subsequently validated using 6 unknown honey samples obtained from the market.

2. Methodology

2.1 Sample Preparation

All pure honeys are collected in Ayer Keroh, Melaka and Pahang directly from the beekeepers and subsequently, subjected to heat treatment at 40^{o}C for 20 minutes to ensure the solid content of honey is fully dissolved. The preparation of adulterated honey from the pure honey is conducted by using corn syrup and domestic sugar as adulterants. The formula 100% – X% is used to prepare the adulterated honey, where X is the weight of adulterant in pure honey. The adulterated honey used in this study is prepared at proportion of 10, 20, 30, 40, 50, 60, 70, 80, 90% of adulterants. Hence, the data set consists of 5 pure honey at each location and 9 different proportion of adulterated honey for each adulterant.

2.2 Spectroscopic analysis

FTIR equipped with Attenuated Total Reflectance (ATR) is used to generate the Infrared (IR) spectrum of honey. The IR spectrum of honey is obtained from 20µl sample of each pure and adulterated honey. The wavelength ranging from 650 to 4000 cm-1 is used to record the spectrum of honey. Sixteen scans are codded with the resolution of 4cm^{-1}. Single-beam spectra of the samples are collected and ratio against the background of air.

The wavelengths between 900 and 1500 cm^{-1} in IR spectrum are used as variables in the research because the region correspond to the presence of sucrose, glucose and fructose in honey [6]. Certain wavelength provides important information regarding the sugar content where table 1 shows several wavelengths with their respective infrared activity of functional group.

Table 1
The IR absorption wavelength and their infrared activity

Wavelength, cm^{-1}	Functional group
110	C-O stretching
918	C-H bending
1043 / 1254	C-O stretching, C-O-H stretching

2.3 Principal Component Analysis (PCA)

The unsupervised pattern recognition is used to categorize data (pattern) based on statistical information obtained from the patterns. In this study, the large data information generated from FTIR spectra is reduced using PCA where redundant information is avoided without losing information from the original data [7]. The objective is to extract important information from the data and represent it in a new orthogonal variable known as principal component (PC). The pattern of similarity of the samples and variables as points in lower dimensional space are displayed by PC [8]. The SOLO+Mia software has been used to run PCA [9].

3. Results and Discussion

Figure 1 illustrates the infra-red spectra of pure and adulterated honeys where variation of absorption of all honeys can be observed. The characteristic of the most important composition of honey range from 750 to 1500 cm^{-1} [6]. Moreover, the concentration of adulterants is also correlated to the wavelength at 750-1500 cm^{-1} since increasing concentration of adulterant affect the spectra at this region. The variation in absorption at wavelength 2700 – 2900 cm^{-1} is attributed to the stretching of C-H bond in carboxylic acids. Nevertheless, this variation is not included in the study because of the low concentration of carboxylic acid in honey [10]. The absorption of O-H group is between 3200 and 3600 cm^{-1}. However, the information in this region can be excluded due to the humidity factor in the room.

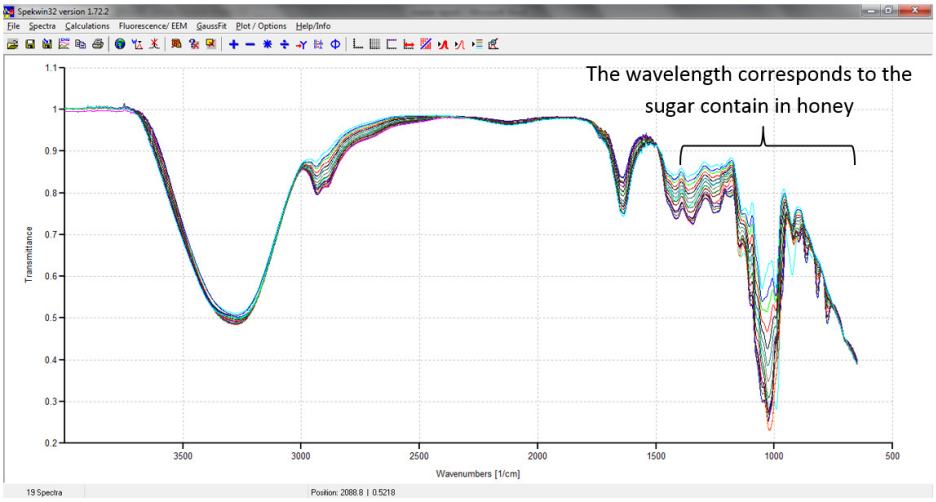

Fig. 1. The FTIR spectra of pure and adulterated honey

Eigenvalue can be used to analyse the number of PC that contributes more information about the model. As depicted in Table 2, PC 1 indicates the highest percentage variance captured which is 86.76 % while PC 2 accounts for 11.16%. The remaining PC only contributes less than 3% of the total percentage variance captured. Since the total percentage of variance captured for the first 2 PC is 97.92%, it can be concluded that these two PC are highly significant and informative in this research. Therefore, PC 1 and PC 2 has been chosen in the classification analysis.

Table 2
The Principal component number with respective % variance captured

Principal Component Number	% variance captured
PC 1	86.76
PC 2	11.16
PC 3	1.28
PC 4	0.58
PC 5	0.22

Based on the score plot illustrated in figure 2, it is obvious that pure honey and adulterated honey with sugar and corn syrup form four distinct groups respectively where PC 1 discriminate between the pure honey and adulterated honeys with sugar and corn syrup. On the other hand, PC 2 indicates classification of pure honey from different location where the pure honey from Pahang is located at the positive value while the pure honey from Melaka is at the negative side of PC 2 axis. Hence, pure honey from Pahang and Melaka is clustered separately in this research because of several factors such as location where honey from Pahang is collected at high altitude in Cameron Highland compared to the honey collected in Ayer Keroh, Malacca where the source of the pollen is different in both places.

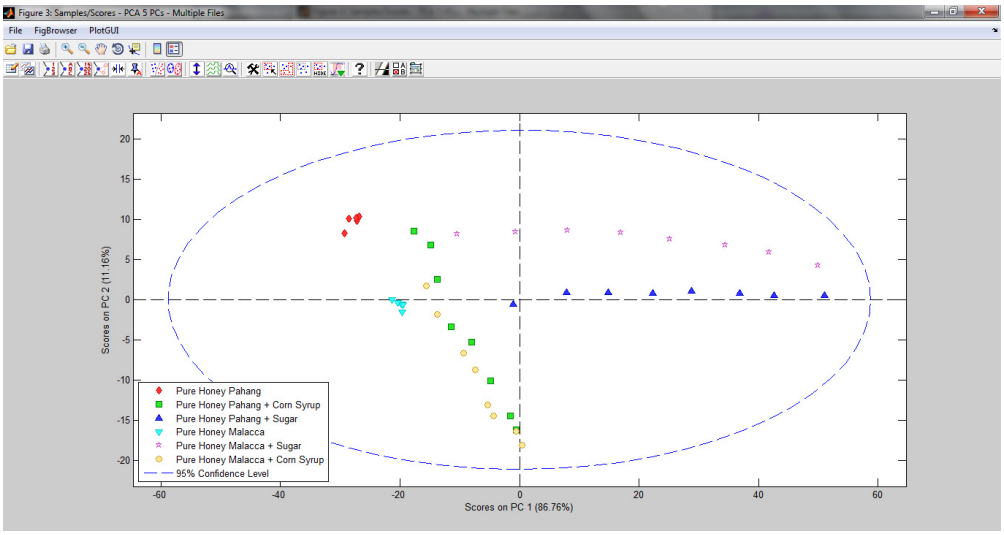

Fig. 2. Score plot (2D) PC 1 vs PC 2

The points representing increasing concentration of sugar is located further to the right or more to the positive side of PC 1 axis in the score plot shown in figure 2. Hence, PC 1 can be used to distinguish between pure honey and their adulteration with sugar. On the contrary, PC 2 is responsible for the cluster of adulterated honey with corn syrup. The adulterated honey with higher concentration of corn syrup is extended towards the negative side of PC 2. This trend is applicable to the adulterated honey from both Pahang and Melaka.

The variables that contribute to the variation in PCA can be determined from the loading plot (figure 3). The wavelength between 1496 and 1500 cm^{-1} region is useful to discriminate the samples for PC 1. The range in 900 – 1100 corresponds to the positive value of PC 2 while 1200 – 1497 cm^{-1} corresponds to the negative value of PC 2.

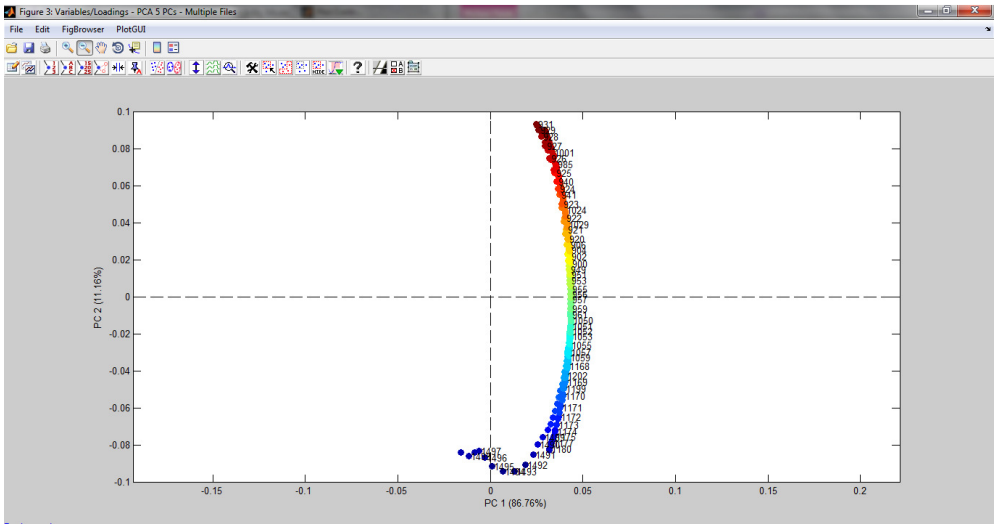

Fig. 3. The loading plot of PCA for honey

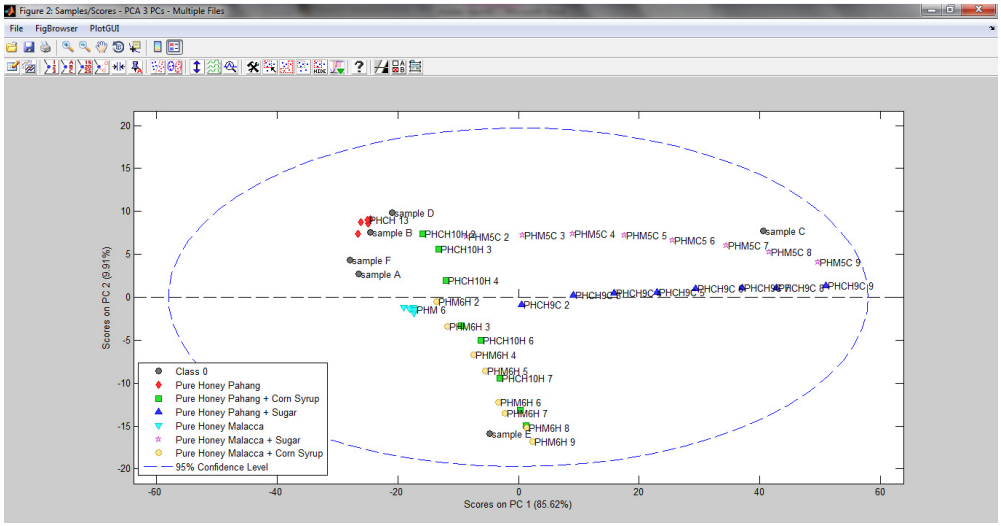

Fig. 4. The score plot of the unknown samples of honey

Consequently, the developed model is further verified using 6 unknown honey samples to determine their purity. Based on the score plot illustrated in figure 4, two unknown samples of honey are suspected not pure because their points are positioned approximately close to sugar and corn syrup group. In this case, sample C is located in the sugar adulterant while sample E is located in the region of corn syrup adulterant region implying that the honey is adulterated with sugar and corn syrup respectively. Both sample C and E are obtained from local market which the seller claimed to be pure honey. The points representing sample A, B, D and F, are located near to the pure honey region from Pahang group and hence suggest that the honeys are pure.

4. Conclusion

The results obtained in the study indicate that the combination of spectroscopic data and chemometric analysis are able to classify different type of honey based on location and purity of honey. Furthermore, this approach can be performed in shorter time and at less cost. In this research, the score plot indicates that the honeys are grouped according to their origins and type of adulterants used. First two PCs with total percentage variance captured approximately 97% are significant to provide sufficient information regarding honey. It is proposed to collect more pure honey from different location in Malaysia and use other different type of adulterant popularly employed in the honey industry. As a conclusion, discrimination of pure and adulterated honey by using chemometric analysis has been successfully performed in this research and can potentially be used as a screening tool to determine the authenticity of honey.

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