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# Analysis of Raw Honey and Syrup Concentration in Adulterated Honey by Fourier Transform Infrared Spectroscopy

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**Abstract**

Adulteration of food is one of the most important issues in quality control and food safety. Therefore, adulteration with sugar syrups, such as corn, cane and cassava syrup, is found in honey in an effort to decrease manufacturing costs. Real honey and adulterated honey are physico-chemical determinations. This study was conducted to predefine types of honey samples for three species (*Apis dorsata* Fabricius, *Apis cerana* Fabricius, *Apis mellifera* Linneaus) from the northern regions of Thailand and to predict the concentrations of cane syrup, cassava syrup and corn syrup in longan honey using attenuated total reflectance-Fourier transform infrared (ATR–FTIR) spectroscopy. Principal component analysis (PCA) was applied to discriminate between pure and adulterated (5 - 50%) honey samples. The partial least squares regression (PLS-R) model was employed to develop the optimal prediction of syrup content in the adulterated honey. The wavelength region that can best classify the Asiatic honey, longan honey, lychee honey and Royal honey samples as belonging to a class is explicitly within the range of 700 - 1600 $cm^{-1}$. The FTIR method successfully provided the quantitative analysis of syrup in longan honey based on the honey's spectrum. However, no significant differences were found among the pH, water, and colour characteristics in raw longan honey and fake longan honey syrup. FTIR is a simple instrument operation with data reproducibility and speed that is waste free and inexpensive per sample when used to determine the purity of different botanical origins and to perform the analysis for the quantification of adulteration of honeys with syrup.

**Keywords**: FTIR-ATR, Honey, Syrup, Authenticity, Botanical origin

## INTRODUCTION

Honey is defined as the natural sweet substance produced by bees from the nectar of plants. It contains unique natural colours, aromas and flavours. Honey is an energy source that has the major carbohydrates by dry weight (glucose, fructose, maltose, sucrose), lesser amounts of water, and a large number of minor components such as proteins, enzymes, amino acids, minerals, trace elements, vitamins, aroma compounds and polyphenols [1].

Fake honey is produced directly by adding syrup and indirectly by feeding bees with syrup. Honey adulteration does not pose significant health problems, but authentication of honey has primary importance for both industries and consumers. Legislation in Europe and Thailand therefore limits the maximum value of sucrose in the finished honey to 5% [2] [3]. Standard honey must not contain corn or cane sugars above a value of 7% according to the AOAC Official Method 998.12 C-4 Plant Sugars in Honey [4].

Many analytical techniques have proved to be able to detect adulterated honey, such as high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), gas chromatography and carbon isotope ratio analysis [5] [6] [7] [8]. Use of these techniques for reliable honey authentication is mostly time consuming and expensive. Fourier transform infrared spectroscopy (FTIR) has been applied to different honey samples for the determination of botanical or geographical origin, detection of adulteration, and quantification of fructose, glucose, sucrose, maltose and sugar syrups [9] [10] [11] [12] [13]. The principle of infrared measurement is based on the detection of different vibrations of atomic bonds caused by excitation with infrared light within a molecule. Since most molecules absorb within this wavelength range, but each has a different structure, differences in the absorption maxima are the result [14]. All criteria to be examined can be determined in one measurement (approx. 1 - 2 minutes) with FTIR. However, this technology requires extensive calibrations in advance to prepare it for a routine analysis of food. For this purpose, all calibration samples must first be examined using standard procedures in order for them to be used as reference samples during the calibration. FTIR spectroscopy has also been used for honey adulteration and characterisation with a limited number of honeys. The objective of this study was to develop a reliable, rapid, cheap and multivariate analysis to quantitatively determine cassava syrup in honey by FTIR-ATR spectroscopy because the past studies were focused on the quantification of

corn and cane syrup. This work was undertaken to determine the physico-chemical properties (moisture content and pH) and colour characteristics of pure honey and honey syrup. In this work, the combination of FTIR spectroscopy and multivariate analysis for detection and determination of the adulteration of longan honey with three types of syrups was investigated.

## MATERIALS AND METHODS

### 3 sets of honey samples

1. Fresh honey samples of three species (*Apis dorsata* Fabricius; 5 Royal honey, *Apis cerana* Fabricius; 5 Asiatic honey, *Apis mellifera* Linneaus; 10 longan honey and 10 lychee honey) were collected from the Department of Agricultural Extension in the Ministry of Agriculture and Cooperatives and the beekeepers of northern regions of Thailand for the physico-chemical properties test. Upon collection, the samples were stored in sterile plastic tubes with a screw cap for analysis.

2. Logan honey in addition to fake (adulterated) honey by dilution with cane syrup, corn syrup and cassava syrup were set in the range between 5%, 10%, 20%, 25% and 50% (w/w) for the calibration and validation with FTIR by each dilution of honey used in 10 samples.

3. Adulterated honey was collected from *Apis mellifera*. Bees in each hive were fed with cane syrup, cassava syrup, and corn syrup in a longan orchard (identified as A1-A3, respectively) for the physico-chemical properties test. Sampling methods were in accordance with the relevant laws and provisions of Thai Agricultural Standard concerning the sampling methods.

### Microbiological test

Ten grams of each honey sample were homogenized in 90 ml of 0.1% peptone water solvent. Decimal dilutions were made into the same solvent until it was 1/1000. Aerobic mesophilic bacteria following protocol (ISO4833:1991) were counted on standard agar count plates (PCA) after being incubated at 37 $^{o}$C for 48 hours. Mould and yeast counts followed the protocol (ISO 21527-2:2008). Microbial counts were expressed as colony-forming units per gram of honey (cfu/g) and performed in duplicate.

### pH determination

The pH of each sample solution containing 5 g of honey and 5 g distilled water was performed in duplicate using a pH meter (Denver: UB-10, Canada). Prior to the measurement, the pH meter was calibrated using three standard buffer solutions, pH 4, 7 and 10 at 25 $^{o}$C.

### Water content

All honey samples were measured for the water content twice with a hand refractometer (ATAGO, HHR-2N, Japan) at 20 $^{o}$C, and reported as a percentage.

### Colour measurement

A colorimeter (Color Quest XE HunterLab, USA) was used to measure the colour parameters, L (brightness), a* (redness), and b* (yellowness) of fresh honey samples. The colorimeter was calibrated using a white reference tile (L* = 93.97, 163 a* = -0.88 and b* = 1.21) and a light trap (black tile) under illumination conditions. The colour of each honey type was measured in 30 replications with the CIELAB system with reference to Illuminant D65 (ASTM) and with a visual angle of 10°. The illuminated area had a diameter of 2.54 mm. Honey samples were placed in cube-shaped containers (5 x 5 x 1 cm$^3$), and colour was measured on each side (in ten replications), at each vertex, and in the middle of each side.

### Spectra acquisition with FTIR Spectrometer

Spectra were collected at 25 $^{o}$C using an Agilent Technologies 4500 FTIR Spectrometer (Malaysia). Thirty-two scans were co-added at a nominal resolution of 4 cm$^{-1}$ in the spectral region of 4000 - 650 cm$^{-1}$. Single beam spectra of the samples were collected and redressed against a background of water and presented in absorbance units. Honey samples were applied at 20 µl. Ten replicas of each sample were recorded at 25 $^{o}$C. The crystal was cleaned between samples with water and dried with methanol and cleaning tissue to eliminate any residues on the surface. Spectra were collected and used for the multivariate analysis, principal components analysis (PCA) and partial least squares regression (PLS-R) by using Unscrambler software (version 9.7, CAMO Software AS, Oslo, Norway). PCA is a bilinear modelling method that gives an interpretable overview of the main information in a multidimensional data table. Data manipulation was processed by taking the second derivative using the Savitzky-Golay algorithm with 9 points of smoothing to minimise the effects of variable baselines and normalised with multiplicative signal correction (EMSC), which normalises spectra, accounting for differences in the samples. Six PCs were chosen for the analysis. By plotting the principle components, one can view the interrelationships between different variables, and detect and interpret sample patterns, as well as group the types of honey. Each sample was replicated ten times. Identical spectra were obtained in each case. The calibration and validation models for the partial least squares (PLS) regression were developed on the basis of the spectral information mainly in the region of 700 - 1600 cm$^{-1}$. All honey samples were stored at 25 $^{o}$C.

### Statistical analysis

All of the tests were performed in triplicate using the SPSS statistical package (SPSS Inc., Chicago, IL), version 18.0. The results were expressed as mean±standard deviation. Testing of the differences among honeys using one-way ANOVA, or the Kruskal Wallis test, and the multiple comparison test with the Duncan method when equal variances were assumed, or the Games-Howell method when equal variances could not be assumed, was conducted to evaluate the significance of differences ($p < 0.05$) among the results.

## RESULTS

The mean bacterial counts of Asiatic honey (10±20 cfu/g), Royal honey (50±20 cfu/g), longan honey (80±20 cfu/g), and lychee honey (20±40 cfu/g) were not significantly different ($p > 0.05$).

Yeast and mould counts obtained from pure honeys (Royal honey, Asiatic honey, longan honey, and lychee honey) were

almost all lower than 10 cfu/g. The adulterated honeys (A1-A3) were lower than 10 - 10 cfu/g for the yeast and mould counts and 10 cfu/g for the mean bacterial counts.

The physico-chemical parameters of the samples, namely, water content, pH determination, and colour measurement, were analysed as shown in Table 1-2.

The pH of pure honey had a minimum value of 3.82 ranging up to a maximum value of 4.48. The water content of pure honey had a minimum value of 17.1% and a maximum value of 19.6%.

**Table 1.** Properties of pure honey

| Type | pH | $H_2O$ | L* | a* | b* |
|---|---|---|---|---|---|
| longan honey | | | | | |
| Sample mean | 4.45 $^{b}$ | 17.37 | 75.81 $^{b}$ | 24.38 $^{c}$ | 68.98 $^{a}$ |
| Sample std. deviation | 0.02 | 0.06 | 0.20 | 0.12 | 0.16 |
| *p*-value of Shapiro-Wilk Test for Normality | 0.463 | 0.000*** | 0.545 | 0.549 | 0.893 |
| Royal honey | | | | | |
| Sample mean | 4.45 $^{b}$ | 17.20 | 74.71 $^{b}$ | 19.93 $^{b}$ | 83.55 $^{c}$ |
| Sample std. deviation | 0.04 | 0.10 | 0.65 | 0.40 | 0.24 |
| *p*-value of Shapiro-Wilk Test for Normality | 0.843 | 1.000 | 0.030* | 0.902 | 0.908 |
| lychee honey | | | | | |
| Sample mean | 3.95 $^{a}$ | 18.33 | 87.26 $^{b}$ | 7.86 $^{a}$ | 65.14 $^{a}$ |
| Sample std. deviation | 0.11 | 1.10 | 3.88 | 2.38 | 12.74 |
| *p*-value of Shapiro-Wilk Test for Normality | 0.085 | 0.174 | 0.270 | 0.926 | 0.106 |
| Asiatic honey | | | | | |
| Sample mean | 4.30 $^{b}$ | 17.55 | 59.24 $^{a}$ | 25.10 $^{c}$ | 77.76 $^{b}$ |
| Sample std. deviation | 0.05 | 0.39 | 0.26 | 0.37 | 0.30 |
| *p*-value of Shapiro-Wilk Test for Normality | 0.878 | 0.122 | 0.789 | 0.000*** | 0.258 |
| *p*-value of Levene's test for Homogeneity of Variances | 0.029* | 0.004** | 0.003** | 0.075 | 0.0012** |
| Test statistic | $H = 9.359$ | $H = 7.594$ | $H = 10.385$ | $H = 10.421$ | $H = 9.462$ |
| *p*-value | 0.025* | 0.055 | 0.016* | 0.015* | 0.024* |

Note: * for $p<0.05$, ** for $p<0.01$, *** for $p< 0.001$, $H$ is the Kruskal-Wallis test's statistic value

**Table 2.** Properties of longan honey syrup (fake honey) and pure honey (longan honey)

| Type | pH | $H_2O$ | L* | a* | b* |
|---|---|---|---|---|---|
| honey syrup (corn); **A1** | | | | | |
| Sample mean | 3.67 | 17.23 | 78.92 [b] | 11.29 [a] | 53.23 |
| Sample std. deviation | 0.03 | 0.06 | 2.02 | 1.10 | 2.34 |
| *p*-value of Shapiro-Wilk Test for Normality | 0.000*** | 0.000*** | 0.547 | 0.955 | 0.766 |
| honey syrup (cassava); **A2** | | | | | |
| Sample mean | 3.30 | 17.42 | 74.02[a] | 15.68 [ab] | 53.03 |
| Sample std. deviation | 0.05 | 0.13 | 0.48 | 1.07 | 1.44 |
| *p*-value of Shapiro-Wilk Test for Normality | 0.567 | 0.780 | 0.919 | 0.295 | 0.053 |
| honey syrup (cane); **A3** | | | | | |
| Sample mean | 3.41 | 18.18 | 74.60 [a] | 19.72 [bc] | 56.62 |
| Sample std. deviation | 0.41 | 1.31 | 2.10 | 7.77 | 20.11 |
| *p*-value of Shapiro-Wilk Test for Normality | 0.047* | 0.036* | 0.953 | 0.128 | 0.032* |
| pure honey (longan honey) | | | | | |
| Sample mean | 4.45 | 17.37 | 75.81 [a] | 24.38 [c] | 68.98 |
| Sample std. deviation | 0.02 | 0.06 | 0.20 | 0.12 | 0.16 |
| *p*-value of Shapiro-Wilk Test for Normality | 0.463 | 0.000*** | 0.545 | 0.549 | 0.893 |
| *p*-value of Levene's test for Homogeneity of Variances | 0.002** | 0.002** | 0.113 | 0.003** | 0.002** |
| Test statistic | $H = 7.642$ | $H = 7.185$ | F = 6.545 | $H = 7.821$ | $H = 3.821$ |
| *p*-value | 0.054 | 0.066 | 0.015* | 0.049* | 0.282 |

Note: * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, *H* is the Kruskal-Wallis test's statistic value, *F* is the F statistic value of One-way ANOVA

The lightness values ($L^*$) of the pure honey samples ranged from 59.21 to 91.69. The Asian honeys demonstrated lower lightness values (59.21). The mean lightness values of the pure honey and longan honey syrup (A1-A3) were 74.26±10.54 and 74.59±2.54, respectively.

The pure honeys showed values between 5.43 and 25.53 for component $a^*$. The mean values of $a^*$ in the pure honey and longan honey syrup (A1-A3) were 18.41±9.50 and 14.79±3.90, respectively. The pure honeys showed values between 50.45 and 83.78 for component $b^*$. The mean values of $b^*$ in the pure honey and longan honey syrup (A1-A3) were 73.86±9.30 and 49.56±8.86, respectively. There were no clearly statistically significant differences among the means of the pure longan honey with each longan honey syrup (A1-A3) in terms of pH, water

content, and the values of $L^*$, $a^*$ and $b^*$, respectively.

The FTIR spectra of the Royal honey, Asiatic honey, longan honey and lychee honey are shown in Figure 1. The first, the selected infrared radiation, was in the average IR range (4000 - 650 cm$^{-1}$). After that, each absorbance was multiplied by the corresponding overall standard deviation. In this way, the spectral ranges that change significantly were accented. Spectra were cut to the 1600 - 700 cm$^{-1}$ range, and they were normalised to unit area. Principal component analysis was applied to the prepared spectra in this way.

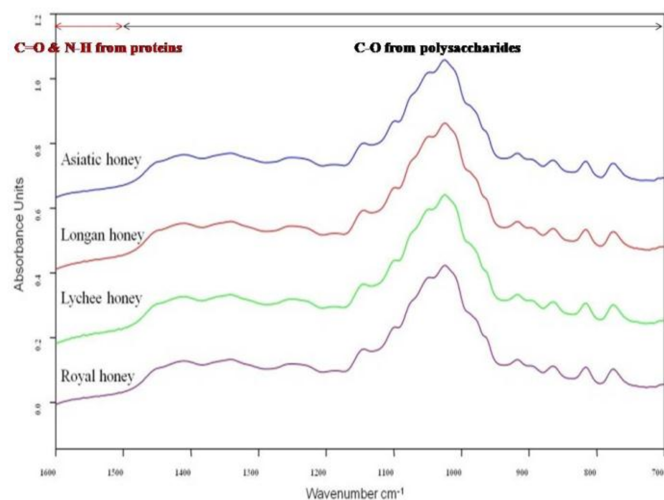
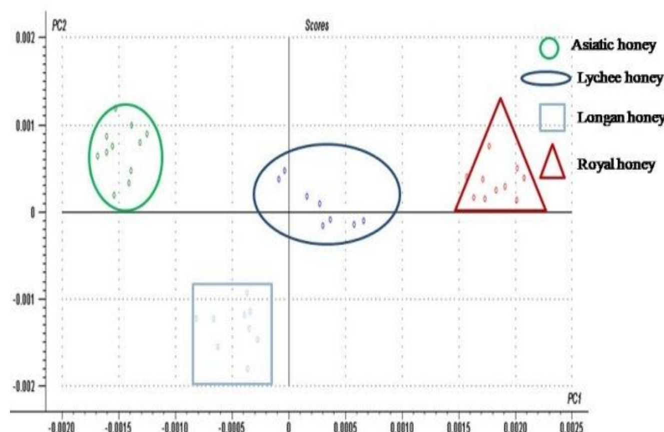
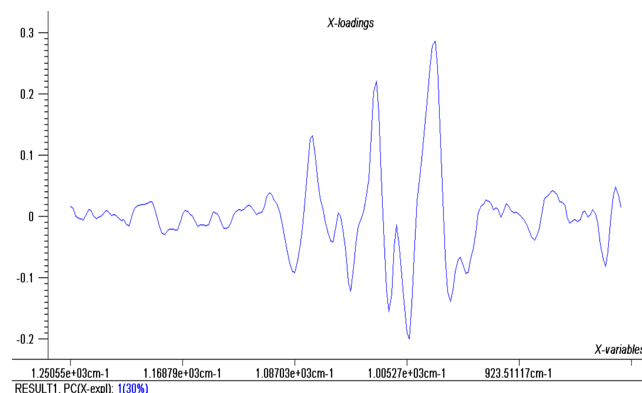



**Figure 1.** The FTIR spectra of Asiatic honey, longan honey, lychee honey and Royal honey

PCA was applied to the spectra of all groups, obtaining an evident discrimination (score plot) of the four different pure honey samples. Figure 2 shows the score plot of the first two principal components PC1 versus PC2 of the second derivative correlation data and the PCA loading plot at the 700 - 1600 cm$^{-1}$ region from the pure honey. The wavelength region that can best classify the Asiatic honey, longan honey, lychee honey and Royal honey samples as belonging to a class is explicitly within the range of 700 - 1600 cm$^{-1}$, covering the region identified as the protein, carbohydrate and fingerprint region of the infrared area.




**(a)**




**(b)**

**Figure 2.** The PCA score plot (a) with the PCA loading plot (b) at the 700 - 1600 cm$^{-1}$ region of the pure honey

Figure 3 shows the score plot of the first two principal components PC1 versus PC2 of the second derivative correlation data from the pure longan honey samples and also from the standard syrup adulterated honey samples (A1-A3). There is a visible agglomeration of the pure honey samples, located in the central zone and the intersection of two well-defined directions of adulteration for each standard syrup in the pure honey. The continuous lines are linear fits of the experimental data and each one of them corresponds to the adulteration of pure honey with each type of syrup. There is a visible trend describing the level of adulteration, with samples belonging to 5% adulteration in the central zone and 50% adulteration at the end of the line. The group of FTIR spectra from all pure honey samples and also the adulterated honey samples with syrups was subjected to PCA, in order to obtain the adulteration directions according to each type of syrup. Fourier transform infrared (FTIR) spectroscopy was used to quantitatively identify (5%, 10%, 20%, 25% and 50% (w/w)) three different adulterants (corn syrup, cassava syrup and cane syrup) in the honey.

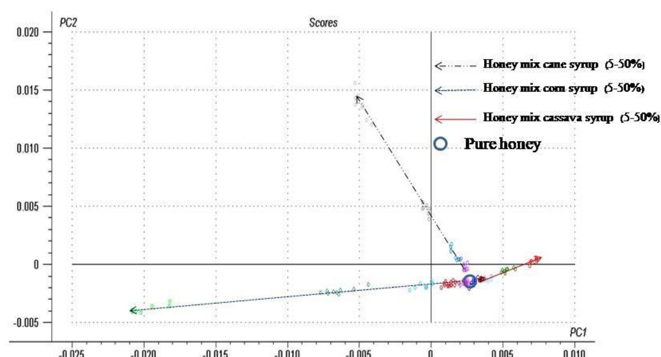



**Figure 3**. The score plot of the first two principal components PC1 versus PC2 of the second derivative correlation data from longan honey (pure honey) samples and also from standard syrup adulterated longan honey samples.

The values of $R^2$, the root mean square error (RMSE) of the optimal calibration models for the types of adulterants (syrups) in honey, are presented by a PLS method in Table 3. The values

of $R^2$ were greater than 0.98 for the calibration data sets. The RMSE of calibration was in the range of 0.983 - 2.680, with 3 being the maximum permitted limit. Validation of the established calibration models with the independent sample showed the $R^2$ values (0.983 - 0.989) and the RMSE (0.982 - 2.565).

**Table 3.** Calibration and validation of the root-mean-square error (RMSE) and R square values for the predicted standard syrups in honey from the PLS model in the 700 - 1600 $cm^{-1}$ region

| Adulterant | Longan honey | | | | Lychee honey | | | |
|---|---|---|---|---|---|---|---|---|
| | Calibration | | Validation | | Calibration | | Validation | |
| | $R^2$ | RMSE | $R^2$ | RMSE | $R^2$ | RMSE | $R^2$ | RMSE |
| Corn syrup | 0.988 | 1.926 | 0.987 | 1.946 | 0.992 | 1.44 | 0.989 | 2.565 |
| Cane syrup | 0.986 | 2.340 | 0.983 | 2.380 | 0.988 | 2.680 | 0.986 | 2.09 |
| Cassava syrup | 0.983 | 0.983 | 0.983 | 0.982 | 0.986 | 1.894 | 0.984 | 2.08 |

## DISCUSSION

All longan honey syrup samples had an average of 10 cfu/g for the bacteria counts and the yeast and mould counts. Bacteria counts obtained from the pure honey samples were all lower than 1.0 x $10^2$ cfu/g and ranged from a low of 10 cfu/g (Asiatic honey) to a maximum of 1.0 x $10^2$ cfu/g in the longan honey. The yeast and mould counts obtained from the pure honeys were almost all lower than 10 cfu/g.

The benchmark limits for Quality Assurance Programs in the Australian honey industry in terms of bacteria count consider the more stringent values, which are lower than 5 x $10^2$ cfu/g and lower than 10 cfu/g for yeast and mould, respectively, as indicators of hygienic management practices [15]. Yeast and mould counts should be less than 10 cfu/g for honey according to the Ministry of Public Health of Thailand and the Codex Alimentarius Commission [2] [3]. Generally, the microbial count in honey may influence its quality or safety. Due to the natural properties of honey and the control measurements of the honey industry, good honey is regarded as a product with minimal types and levels of microbes (similar to the pure and fake honey results of this study).

The mean pH values of the pure honey and fake honey were 4.28±0.22 and 3.33±0.22, respectively. These values of the pure honey are similar to those previously reported that were found to be 3.50 - 4.58 [16] [17] [18] [19]. Another important parameter during the extraction and storage of honey is the pH value, which influences honey texture, stability and shelf life [20].

The mean water content of the pure and fake honey was 17.61±0.67% and 18.84±1.39%, respectively. Evidently, these samples are within the range of the standards that require the water content to be lower than 21%, according to the International Regulations of Quality [3]. These results are similar to those previously reported for these monofloral honeys [17] [21] [22]. Conspicuously, the water content of honey depends on various factors, such as the harvesting season, the degree of maturity achieved in the hive, the geography, the botanical origin, processing techniques, and storage and environmental factors [23] [24] [25].

The analysed pure honeys and honey syrup also contained the most common characteristics of being bright, light coloured honey and possessed both yellow and red components. All samples had light tones. The lightness of the ingredients plays a major role in the honey assessment due to consumer preferences. Pure honey results in a significant clarity of all colour values. The CIE L*a*b* is chosen by food industry professionals because its system correlates well with how the human eye perceives colour. The colouring of honey acts as the first essential character that customers consider. The composition and colour of honey depend mainly on the flowers, geographical regions, climate and honeybee species involved in its production, and are also affected by weather conditions, processing, manipulation, packaging and storage time [26] [27]. The Codex Alimentarius Committee on Sugars stipulates that the colour of honey should range from nearly colourless to dark brown [3]. Thus, the characteristics of honey are the biological factors, colour, pH, and water content, which were not indicators of clearness when classifying pure longan honey and each longan honey syrup (A1-A3).

The comparative spectral features seen in Figure 1 of the four pure honeys in relation to the average spectra of the spectral bands in the sample were corresponding to previous reports [28] [29] of the whole spectral from 4000 - 600 $cm^{-1}$ and the spectral region between 1600 - 700 $cm^{-1}$, the most variable part of honey spectrum populated by the most prominent strong absorption band. The wavelength region that can best classify the Asiatic honey, longan honey, lychee honey and royal honey samples as belonging to a class is explicitly within the range of 700 - 1600 $cm^{-1}$.

The assignment of each functional group corresponding to the vibration modes was based on the designation of the spectrum peaks and frequency matching with the corresponding chemical group that is absorbed in the IR regions. The region of 700 - 1500 $cm^{-1}$ corresponds to the absorption zones of the three major sugar constituents of honey [30]. The bands in the approximate region of 1600 $cm^{-1}$ had been formerly assigned as amide I protein vibrations. Proteins are a negligible constituent in honey; however, they are used in detecting adulteration [31].

Honey is susceptible to adulteration with cheaper sweeteners or by an indirect approach of feeding bees with sugar or syrup. Therefore, methods for detecting and quantifying adulteration are necessary. Adulteration of pure honey with synthetic honey (based on C4 plant sugars) has become much more prevalent in recent years [32]. In addition, there has been a recent major problem of adulteration of honey with syrup. At present, a variety of analytical techniques have been developed to detect adulteration of honey. Some of these methods, such as stable carbon isotope radio analysis, are time-consuming, and others are expensive. Although there are powerful methods to prove honey adulteration, they must be further improved in order to ensure honey quality. The limited availability and high price of honey have provided a heightened interest in its adulteration. The identity and quality parameters of honey are considered

useful for detecting these possible adulterations, and also for confirming the hygienic conditions for the manipulation and storage of honey. Therefore, Fourier transform infrared (FTIR) analysis is an ideal technique for the quantification of honey and the quantitative analysis of honey syrup. The limitations of the FTIR technique are the high-priced tools and high maintenance costs.

## CONCLUSIONS

Honey is a natural sweet substance produced by honeybees from the nectar of flowers or other living parts of plants which the bees collect, naturally transform and store in the honeycomb. Given its composition (mainly carbohydrates and water), honey is adulterated in practice by cheaper, commercially available sugar syrups with similar composition. It is an important parameter in the quality of the honey, as well as the acceptance and preference of consumers. Observation with the naked eye and biological and physical tests are unable to distinguish raw honey from honey syrup.

The adulteration of honey is a serious economic and regulatory problem. Fourier transform infrared (FTIR) spectroscopy was used to quantitatively identify three different adulterants (corn syrup, cassava syrup and cane syrup) in honey and help to visualise the class separation among honey samples of three species (*Apis dorsata* Fabricius; Royal honey, *Apis cerana* Fabricius; Asiatic honey, *Apis mellifera* Linneaus; longan honey and lychee honey). This study is the first work that has reported that FTIR spectroscopic analysis is able to qualitatively and quantitatively determine the adulterant (cassava syrup) in honey. Fourier transform infrared (FTIR) spectroscopy offers a fast, non-destructive alternative to chemical measurement techniques for qualitative characterisation and quantification of the majority of syrup content in honey, and it is easy to adapt in order to be used as a method in the analysis of honey.

## CONFLICTS OF INTEREST

There is no conflict of interest in this study.

## ACKNOWLEDGEMENT

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## REFERENCES

[1] Bogdanov S., Jurendic T., Sieber R., Gallmann, P. (2008). Honey for nutrition and health: a review. *Journal of the American College of Nutrition*. 27(6):677-689.

[2] Ministry of Public Health, The Royal Thai government. (2000). Honey. The declaration of the Ministry of Public Health No. 211.2000 (B.E. 2543).

[3] Codex Alimentarius. (2001). Revised 2 Codex Standard for honey. Codex Alimentarius, Rome. 12: 1-7.

[4] AOAC International. (1999). AOAC Official Method 998.12. C-4 plant sugars in honey. In: Cunniff, P.(ed). Official Methods of Analysis of AOAC International. Gaithersburg. p. 27–30.

[5] Wang S., Guo Q., Wang L., Lin L., Shi H. H., Cao H., Cao B. (2015). Detection of honey adulteration with starch syrup by high performance liquid chromatography. *Food Chemistry*. 172: 669-674.

[6] Zhu X., Shan Y.Li.S., Shan Z., Su D.Li.G., Liu, F. (2010). Detection of adulterants such as sweeteners materials in honey using near-infrared spectroscopy and chemometrics. *Journal of Food Engineering*. 101:92-97.

[7] Bertelli D., Lolli M., Papotti G., Bortolotti L., Serra G., Plesi, M. (2010). Detection of honey adulteration by sugar syrups using one-dimensional and two-dimensional high-resolution nuclear magnetic resonance. *The Journal of Agricultural and Food Chemistry*. 58:8495-8501.

[8] Fei X., Wu B., Sehn C., Ding T., Ti L.,Lu Y. (2011). Honey adulteration detection using liquid chromatography/ elemental analysis-isotope ratio mass spectrometry. *Chinese Journal of Chromatography*. 29:5-19.

[9] Chung H., Ku S.M., Lee S.L. (1999).Comparison of near-infrared and mid infrared spectroscopy for the determination of distillation property of kerosene. *Vibrational Spectroscopy*. 20(2):155–163.

[10] Lichtenberg-Kraag B., Hedtke C., Bienefeld K. (2002). Infrared spectroscopy in routine quality analysis of honey. *Apidologie*. 33(3):327–337.

[11] Ruoff K. (2006). Authentication of the botanical origin of honey. MSc Dissertation. Swiss Federal Institute of Technology. Zurich, Switzerland.

[12] Rios-Corripio M.A., Rojas-Lpez M., Delgado-Macuil R. (2012).Analysis of adulteration in honey with standard sugar solutions and syrups using attenuated total reflectance-Fourier transform infrared spectroscopy and multivariate methods. *CYTA–Journal of Food*. 10(2):119–122.

[13] Kelly J.D., Downey G., Fouratier, V. (2004). Initial study of honey adulteration by sugar solutions using midinfrared (MIR) spectroscopy and chemometrics. *The Journal of Agricultural and Food Chemistry*. 52:33–39.

[14] Li-Chan E., Chalmers M.J., Griffiths P. (2010). Applications of vibrational spectroscopy in food science. Volume 1: Instrumentation and fundamental applications. John Wiley & Sons, Chichester.

[15] Ward H.W., Trueman F. K. (2001). A quality survey of Australian honeys. A report for the Rural Industries Research and Development Corporation. Queensland Department of Primary Industries. RIRDC Publication No 01/049.

[16] Rashed N.M., Soltan E.M. (2004). Major and trace elements in different types of Egyptian mono-floral and non-floral bee honeys. *Journal of Food Composition and Analysis*. 17:725–735.

[17] Fea´s X., Pires J., Iglesias A., EstevinhoL. M. (2010). Characterization of artisanal honey produced on the Northwest of Portugal by melissopalynological and physico-chemical data. *Food and Chemical Toxicology*. 48:3462–3470.

[18] Ouchemoukh S., Louaileche H., Schweitzer P. (2007). Physicochemical characteristics and pollen spectrum of some Algerian honeys. *Food Control*. 18:52-58.

[19] Kanyaluck J., Yingmaneea, T. (2015). Activities of different types of Thai honey on pathogenic bacteria causing skin diseases, tyrosinase enzyme and generating free radicals. *Biological Research*. 48(4)**:**1-11.

[20] Terrab A., Dıez M.J.,Heredia F.J. (2003). Palynological, physico-chemical and colour characterization of Moroccan honeys: I. River red gum (Eucalyptus camaldulensis Dehnh) honey**.** *International Journal of Food Science* and *Technology*. 38:379-386.

[21] Manzanares B. A., Garcı´a H.Z., Galdo´n R.B., Rodrı´guezR.E., Romero, D.C. (2011).Differentiation of blossom and honeydew honeys using multivariate analysis on the physicochemical parameters and sugar composition. *Food Chemistry*. 126:664–672.

[22] Karabagias K.I., Badeka A., Kontakos S., Karabournioti S., Kontominas G.M. (2014). Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics. *Food Chemistry*. 46: 548–557.

[23] Acquarone C., Buera, P.,Elizalde, B. (2007). Pattern of pH and electrical conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys. *Food Chemistry*. 101: 695–703.

[24] Anklam, E. (1998).A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry*. 63:549– 562.

[25] Yücel Y., Sultanoglu P. (2013). Characterization of honeys from Hatay region by their physicochemical properties combined with chemometrics. *Food Bioscience*. 1:16–25.

[26] Escuredo O., Dobre I., Fernández-González M.,Seijo C.M. (2014). Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chemistry*. 149: 84–90.

[27] Tornuk F., Karaman S., Ozturk I., Toker S.O., Tastemur, B., Sagdic, O., Tastemur, B., Dogan, M., Kayacier, A. (2013). Quality characterization of artisanal and retail Turkish blossom honeys: Determination of physicochemical, microbiological, bioactive properties and aroma profile. *Industrial Crops and Products*. 46:124–131.

[28] Gallardo-Velázquez T., Osorio-Revilla G., Loa Z.M., Rivera-Espinoza Y. (2009). Application of FTIR-HATR spectroscopy and multivariate analysis to the quantification of adulterants in Mexican honeys. *Food Research International*. 42:313-318.

[29] Machana S., Ornnim P., Weeakul T., Ninsanthea C.,PhanudulkottiC., Srijan T., PattarapanichC., Thumznu K., Tanthanuch, W. (2012).The study of application for discrimination of royal honey bee from three Thai stingless honey bees (*Trigona* spp.) using FTIR-microspectroscopy. *Thai Journal of Pharmaceutical Sciences*. 36(Suppl.):193-196.

[30] Tewari J., Irudayaraj, J. (2004). Quantification of saccharides in multiple floral honeys using transform infrared microattenuated total reflectance spectroscopy. *The Journal of Agricultural and Food Chemistry*. 52:3237–3243.

[31] Arvanitoyannis I.S., Chalhoub C., Gotsiou P., LydakisSimantiris N., Kefalas P. (2005). Novel quality control methods in conjunction with chemometrics (multivariate analysis) for detecting honey authenticity. *Critical Reviews in Food Science and Nutrition*. 45(3): 193-203.

[32] White W.J., Winters K., Martin P., Rossmann, A. (1998). Stable carbon isotope ratio analysis of honey: Validation of internal standard procedure for worldwide application. *Journal of AOAC International*. 81: 610-619