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Determination and Analysis of Fake Honey and Stingless Bee Honey

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GRAPHICAL ABSTRACT

Honey pot of stingless bee honey from UTM's farm

ABSTRACT

Recently, there has been a report in local newspaper about fake honey that has been widespread sold in the market. There are several types of honey that has been adulterated especially stingless bee honey, since a high demand from consumers and owing to its nutritional and benefits to health. Besides, the production of stingless bee honey itself is 50 times less than regular honey bees. Thus, the production of fake honey with addition of sugar that has been adulterated to pure honey is out of control solely to make an extra buck. In this study, the samples used were pure stingless bee honey from Universiti Teknologi Malaysia's (UTM) farm, local honey products and sugar from market. The local honey samples were analyzed of their sucrose and made comparison with laboratory prepared sucrose from sugar and pure stingless bee honey. Determination and analysis of pure stingless bee honey and fake honey also sugar samples were carried out using attenuated total reflectance Fourier Transform infrared (ATR-FTIR), ultraviolet-visible (UV-Vis) and nuclear magnetic resonance (NMR) spectroscopy. Qualitative analysis of pure, samples of stingless bee honey and sucrose using ATR-FTIR showed a difference between pure honey, samples of honey and sugar at the bands between 1400 to 750 cm^{-1} correspond to the most sensitive absorption region of honey sugar. Moreover, the honey samples were suspected to contain sucrose, and it was supported by the analysis using UV-vis spectroscopy that showed a peak at 280 nm to represent sucrose. Meanwhile, the analysis using NMR spectroscopy showed a difference at chemical shift 4.2 to 3.0 ppm. In summary, this study shows that local honey samples contain sucrose.

Keywords: stingless bee honey, fake honey, sugar addition, ATR-FTIR spectroscopy, NMR spectroscopy

1. INTRODUCTION

Over the past 2,500 years ago honey has been used by countless cultures all around the world. It has been used both as food and medicine since ancient times. The primary composition of the honey was fructose and glucose in the form of fructo-oligosaccharides [1]. Another dominant composition of honey was amino acids, vitamins, minerals and enzymes [2].

Among others type of honey, stingless bee honey has strong anti-bacterial & anti-toxin function, dilation of blood vessels, strengthen our immune system and activation of cells. Moreover, stingless bee honey contains natural antibiotic elements and function as antioxidant. In short, stingless bee honey is twice as nutritious as ordinary honey, according to the Malaysian Agricultural Research and Development Institute (MARDI) [3].

The price of natural honey is much higher than other sweeteners making it susceptible to produce fake honey using addition any types of sugar. However according to the Food Act 1983, any commercially available "pure" labelled honey products that are found to have in excess of 10% by weight of sucrose or maltose are considered to be adulterated [4]. The most common adulterants are sweeteners, such as sugar cane, high-fructose corn syrup (HFCS), beet invert syrup and maltose syrup (MS). Such adulteration has a negative impact on both authentic honey producers and consumers. In this study, for determination of analysis of fake honey as qualitatively method it used to be compared with pure honey and sugar itself and focused at region of sugar profiling.

This research will emphasize on determination and characterizing pure stingless bee honey, sugar and honey from local market as a sample. The method to identify fake honey, to detect the presence of sugar which majority component of sugar is sucrose in local honey qualitatively by using attenuated total reflectance Fourier Transform infrared (ATR-FTIR), ultraviolet-visible (UV-Vis) and nuclear magnetic resonance (NMR) spectroscopy respectively.

2. EXPERIMENTAL

In this chapter, there are three major parts of the study. First of all, the pure stingless bee honey was obtained from Universiti Teknologi Malaysia (UTM)'s farm. Then, two sample randomly bought at local market that be labelled as Sample 1 and Sample 2. Besides, the preparation of sugar was preparedly in laboratory by heating on a hot plate until its melt. Before the analysis, the samples were stored in a dark place at the room temperature.

The first part of this study was by qualitatively determination the pure honey, honey samples and sugar by FTIR-ATR spectroscopy (Frontier, Perkin Elmer) by comparing the spectrum obtained and the percentage transmittance measure in the range 650 to 4000 nm. Secondly, the study proceeded with an analysis the presence of sucrose by UV-Vis spectroscopy (Shimadzu UV-1800). At this part, the sample was diluted with 50 mL of methanol solution and adjusted with distilled water until concentration of mixture 5000 μg/mL. Later, in last part, the pure honey, honey samples and sucrose were analyse to study their profiling composition by using NMR spectroscopy (Bruker, 400MHz) D_2O as solvent and predicted at ^1H NMR chemical shift.

3. RESULTS AND DISCUSSION

3.1. Analysis of Stingless Bee Honey by Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

In the current study, ATR-FTIR spectroscopy has been used to compare honey samples based on their spectral differences in the 4000–650 cm^{-1} spectral region. A representative ATR-FTIR spectrum of pure honey is given in Figure 1. Table 1 presents the band assignments along with the corresponding modes of vibrations in the ATR-FTIR spectrum of honey, based on the literature [5].

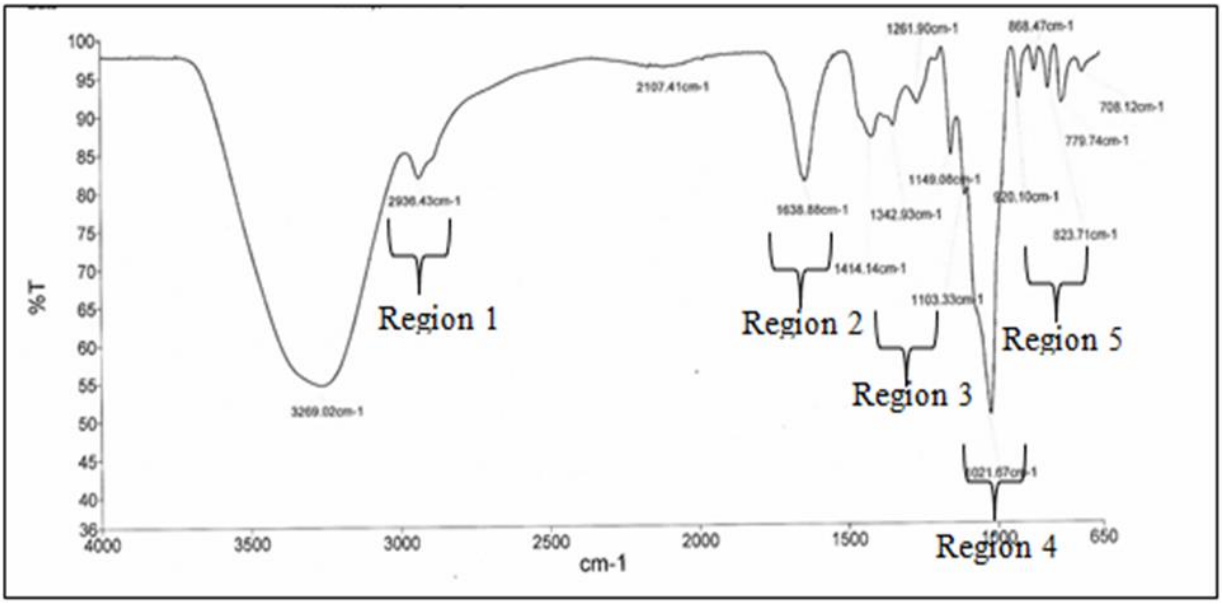

Figure 1 ATR-FTIR spectrum of standard stingless bee honey.

Table 1 General bands assignment of ATR-FTIR spectrum of honey

Region	Wavelength	Bands
Region 1	3000-2800 cm^{-1}	C-H stretching (carbohydrates)
Region 2	1700-1600 cm^{-1}	O–H stretching/bending (water)
		C=O stretching (mainly from carbohydrates
Region 3	1540-1175 cm^{-1}	O–H stretching/bending
		C–O stretching (carbohydrates)
		C–H stretching (carbohydrates)
Region 4	1175-940 cm^{-1}	C–O & C–C stretching (carbohydrates)
		Ring vibrations (mainly from carbohydrates)
Region 5	940-700 cm^{-1}	Anomeric region of carbohydrates
		C–H bending (mainly from carbohydrates)
		Ring vibrations (mainly from carbohydrates)

Figure 2 shows comparative infrared spectra between pure stingless bee honey, honey samples and sucrose in the 4000–650 cm^{-1} region. In this figure, spectral differences between the groups were clearly seen. The characteristic bands of major monosaccharides absorption (glucose, fructose) and disaccharide (sucrose) were present in the region between 1400 and 900 cm^{-1}. Thus, sample 1 and sample 2 might be suspected as a fake honey.

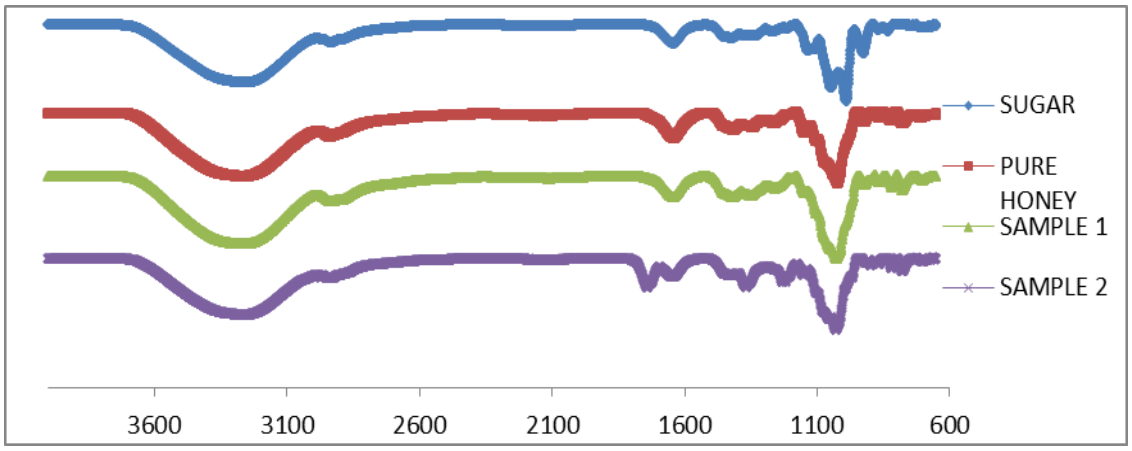

Figure 2 Comparative ATR-FTR spectra of all samples in the 4000–650 cm^{-1} spectral region.

3.2 Quantitative Analysis of Sucrose by Ultraviolet Visible Spectroscopy

The maximum UV-Vis absorbance of sucrose will be expected to show at wavelength of 200 - 350 nm. In this part, study used to make comparison between pure stingless bee honey and two samples from the local market. The representative of UV-Vis spectrum is shown in Figure 3 below.

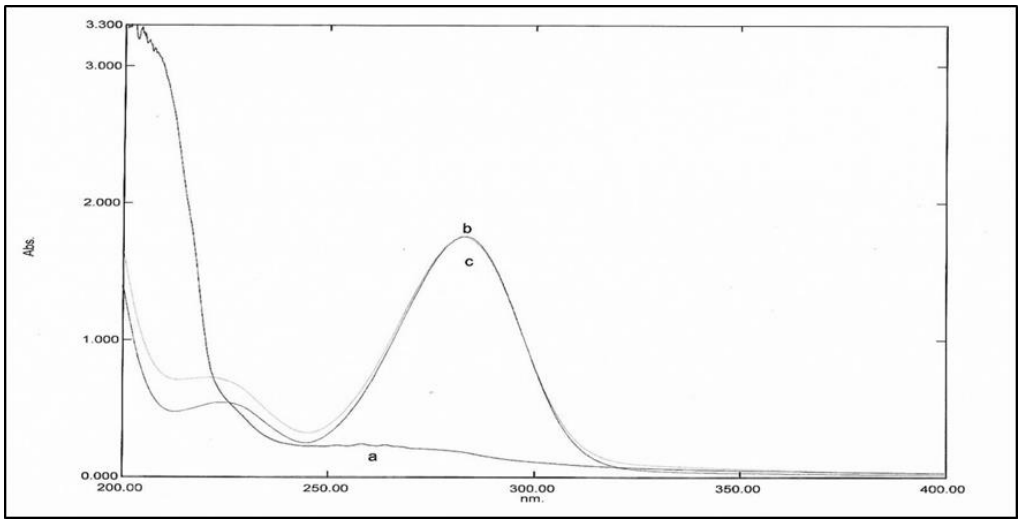

Figure 3 UV-Vis spectrum of (a) pure stingless bee honey (b) sample 1 (c) sample 2.

Based on the Figure 3, analysis of sucrose in local honey using UV-Vis spectroscopy gives the absorbance of sucrose at 280 nm of wavelength. The peak (a) which referred to pure stingless bee honey indicated no peak form 200 nm-350 nm. Meanwhile, sample 1(b) and sample 2(c) indicated a peak 283.60 nm and 282.80 nm respectively. The absorbance reading for sample 1 was 1.757 and sample 2 was 1.756.

3.3 Qualitative Analysis of Stingless Bee Honey by Nuclear Magnetic Resonance Spectroscopy

For ^{1}H NMR of standard stingless bee honey, honey sample from local market and sugar component will be expected to show the chemical shifts δ in ppm. The honey is expected to show chemical shifts at around δ 4.2 and 3.0 ppm which is dominated with very intensive signals of the major monosaccharides (glucose and fructose) and disaccharides (maltose and sucrose). Figure 4 until 7 was expanded the region of the peak, chemical shift 3.0-4.2 ppm, 5.0-5.2 ppm and 4.45-4.55 ppm. This expended region to determination of sugar parts as represented at Table 2 [6].

Table 2 Analytical Characteristics of the Quantitative Determination of Glucose, Fructose and Sucrose

	Chemical shift used for quantification (ppm)
Glucose	5.23 (α-glucose)
	3.24 (β-glucose)
Fructose	4.1
Sucrose	4.22

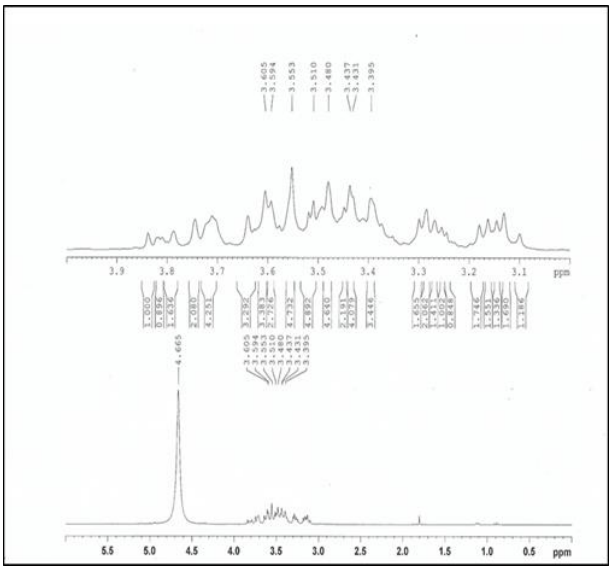

Figure 4 NMR spectrum of Pure Stingless Bee Honey.

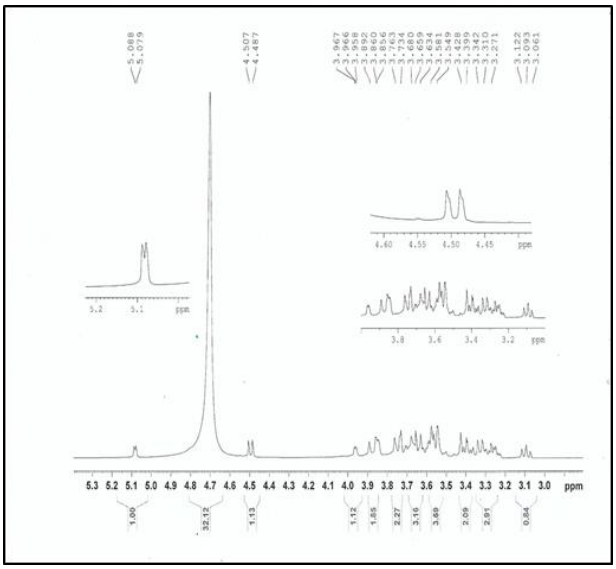

Figure 5 NMR spectrum of Sugar.

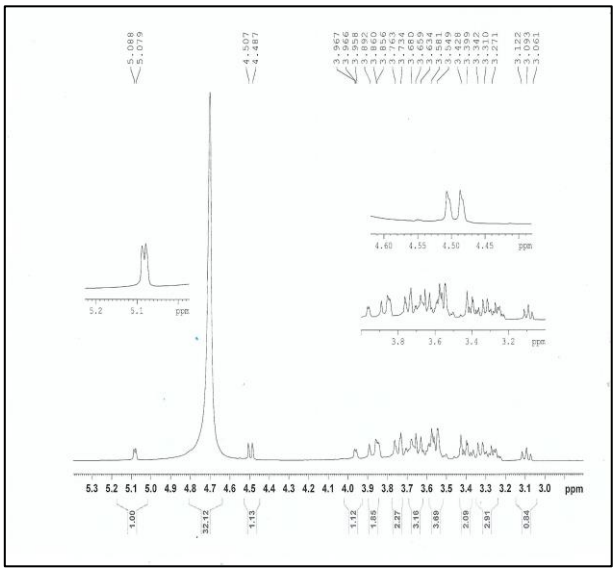

Figure 6 NMR spectrum of sample 1 of Honey.

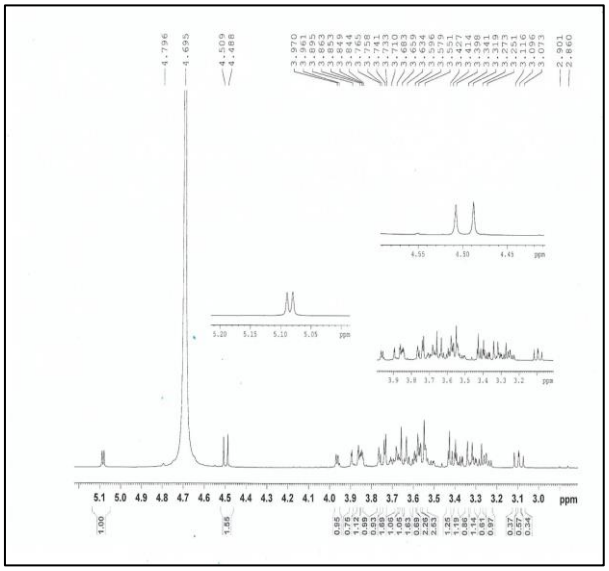

Figure 7 NMR spectrum of sample 2 of Honey.

Table 3 Result Characteristic of the Quantitative Determination of Glucose, Fructose and Sucrose Chemical Shift (ppm)

	Pure honey	**Sugar**	**Sample 1**	**Sample 2**
Glucose	3.3 ppm (β-glucose)	3.56 ppm (β-glucose) 5.29 ppm (α-glucose)	3.25 ppm (β-glucose) 5.09 ppm (α-glucose)	3.42 ppm (β-glucose) 5.08 ppm (α-glucose)
Fructose	-	4.09 ppm	4.48ppm	4.48 ppm
Sucrose	-	4.11 ppm	4.51ppm	4.50ppm

4. CONCLUSION

Through this work, we had successfully employed three spectroscopic methods in analysis and identifying pure stingless bee honey and fake honey qualitatively and detecting the presence of sucrose. This analysis was done by ATR-FTIR, NMR and UV-Vis spectroscopy and these proposed methods are simple, easy in handling and cost-effective to analyse stingless bee honey and for adulteration of local honey with sweetener. The development of the effective UV-Vis spectroscopy in screening, detection and confirmatory of sucrose hopefully can help the enforcement agencies to do inspection on the products available in the market, and thus can reduce illegal business of fake honey. As conclusion, all these analysis methods using ATR-FTIR, UV-Vis, NMR spectroscopy proved that it is able to identify fake honey and quantify the presence of sucrose in honey samples. The obtained spectrums gave major information in determination and quantification of the presence of sucrose.

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