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Nutritional Analysis of Stingless and Stinging Bee Honey by Spectroscopic Methods

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GRAPHICAL ABSTRACT

Stinging bee honey (left) and stingless bee honey (right)

ABSTRACT

Honey has been used as both sweetener and medicine since ancient times. Recently, there has been a report regarding stingless bee honey where it is twice as nutritious as stinging bee honey. Besides that, according to Food Act the percentage of sucrose in honey should be less than 10%. In this study, samples of melted sugar, stingless and stinging bee honey were collected and analyzed using Fourier Transform Infrared Attenuated Total Reflectance (FTIR-ATR) spectroscopy, Ultraviolet-Visible (UV-Vis) spectroscopy and Nuclear Magnetic Resonance (NMR) spectroscopy. Qualitative analysis of stingless and stinging bee honey by FTIR-ATR showed differences at the band of 1175 cm^{-1} to 1700 cm^{-1} indicating lesser intensity than sugar. Intensity is affected by analyte concentration which proves that sugar contains higher concentration of sucrose. Moreover, sugar showed the highest absorbance of 0.307 at 278 nm which is supported by the analysis using UV-Vis to represent sucrose. Sucrose is made up of glucose and fructose. The analysis of NMR spectroscopy showed sugar having strong signal at 5.2 ppm indicating glucose which is absent in both honey samples. Meanwhile, fructose is present in stingless bee honey at 4.1 ppm while absent in stinging bee honey. The study shows that stingless bee honey is more nutritious than stinging bee honey in terms of sucrose content.

Keywords: stinging bee honey, stingless bee honey, FTIR-ATR spectroscopy, UV-vis spectroscopy, NMR spectroscopy.

1. INTRODUCTION

Honey is a floral extract and secretions from various bees [1]. Honeys are classified into two groups namely honeybee and stingless bee [2]. The honeybee is large in size and it stings while the stingless honeybee is smaller and does not stings [3]. Honeybee honey tastes sweet, but the stingless honey is a mixture of sweet and delicate taste [4]. Sugar are composed of carbon, hydrogen and oxygen. There are various types of sugars such as fructose, galactose, lactose and maltose derived from different sources.

This research is to identify and differentiate between pure stingless bee honey and pure stinging bee honey using spectroscopic methods such as Fourier Transform Infrared Attenuated Total Reflectance (FTIR-ATR) spectroscopy, Ultraviolet-Visible (UV-Vis) spectroscopy and Nuclear Magnetic Resonance (NMR) in terms of nutritional content. Through this study, spectrum of both honey types is analyzed quantitatively and qualitatively. Quantitative analysis will be done on the honey samples and compared with sugar used as standard whether the percentage of sucrose present in those honey samples are above or below 10%. Qualitative analysis will be done on the honey samples to identify types of sucrose derivatives present in each honey. This research helps to have a strong consumer demand due to the benefits of nutritional content in terms of sucrose and able to overcome and reduce health problems such as diabetes and cancer.

2. EXPERIMENTAL

The experiment was divided into two main parts which were to identify the nutritional content in terms of sucrose in stingless bee honey which makes it more nutritious than stinging bee honey and to compare the sucrose content in the honey samples with sugar as standard. Firstly, pure stingless bee honey and pure stinging bee honey were collected from respective farms. Meanwhile, white sugar was purchased from market and heated till melt on a hot plate. The honey samples and sugar were characterized by FTIR-ATR spectroscopy, UV-Vis spectroscopy and NMR spectroscopy. The spectrums obtained were analysed quantitatively and qualitatively.

3. RESULTS AND DISCUSSION

3.1. Analysis of pure stingless and pure stinging bee honey using (FTIR-ATR) spectroscopy

FTIR-ATR were used to compare honey samples and standard sugar based on their spectral differences in the 1175 cm^{-1} to 1540 cm^{-1} spectral region. A representative FTIR-ATR spectrum of melted white sugar is shown in Figure 1.

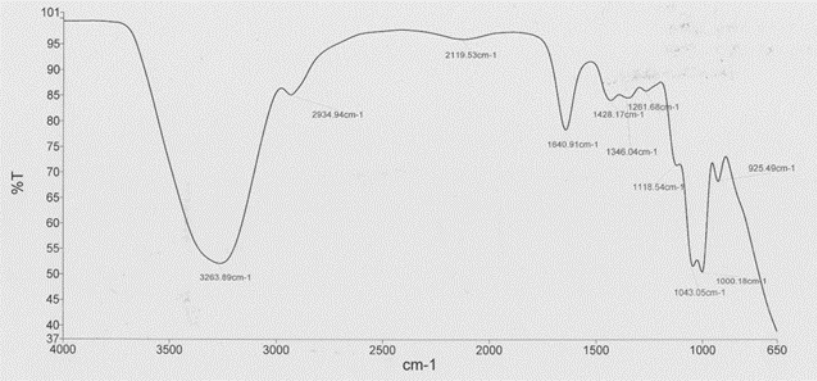

Figure 1. FTIR-ATR spectrum of melted white sugar standard

The composition of sugar has a major infrared band of water located at 3263.89 cm^{-1} for O-H stretching and 1640.91 cm^{-1} for H-O-H bending vibrations. The peak at 918 cm^{-1}, 1043 cm^{-1} and 1254 cm^{-1} corresponds to the C-H bending of the carbohydrate, C-O stretch in the C-OH group as well as the C-C stretch in the carbohydrate structure, respectively. Similarly, other studies reported that spectral region located between 1500 cm^{-1} and 750 cm^{-1} was characteristic of the carbohydrate configuration [5]. As shown in Figure 1, the peak located at 1026.34 cm^{-1} corresponds to stretching of the C-O band of the C-O-C linkage while the peak that appeared at 1254.84 cm^{-1} was due to the O-H bending of the C-OH group. The broad band located between 3000 cm^{-1} and 2800 cm^{-1} corresponds to the C-H stretching of sucrose [6].

From Figure 2 and 3, the spectrums of pure stingless and pure stinging bee honey showed differences with spectrum of standard melted white sugar at band 1175 cm^{-1} to 1700 cm^{-1}. Pure stingless and pure stinging bee honey showed lesser intensity overall than spectrum of standard sugar. There is no peak seen at 1110 cm^{-1} in both pure stingless and pure stinging bee honey spectrums. Intensity is affected by analyte concentration which proves that sugar contains higher concentration of sucrose.

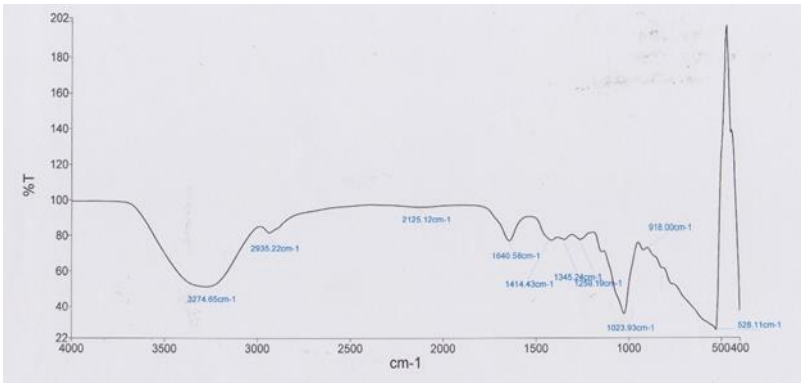

Figure 2. FTIR-ATR spectrum of pure stingless bee honey

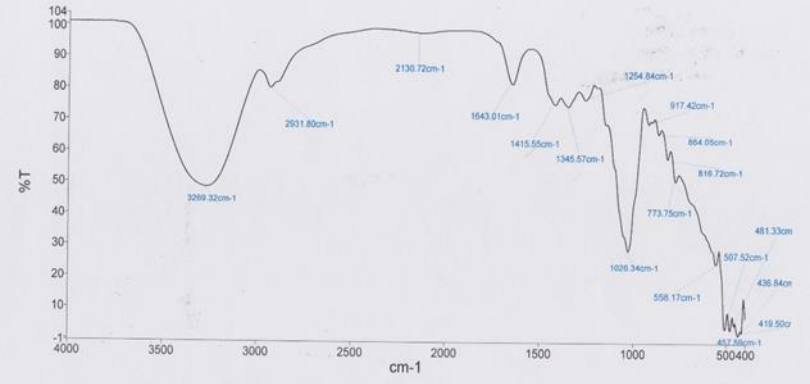

Figure 3. FTIR-ATR spectrum of stinging bee honey

3.2. Analysis of pure stingless and pure stinging bee honey using UV-Vis spectroscopy

UV-Vis were used to compare honey samples and standard sugar based on their absorbance of sucrose. The maximum UV-Vis absorbance of sucrose, pure stingless and pure stinging bee honey were expected to show at wavelength of 200 nm to 400 nm. The representative of UV-Vis spectrum is shown in Figure 4. Figure 4 shows spectrum of pure stingless bee honey, pure stinging bee honey and standard sugar. Stingless bee honey sample showed absorbance of 0.065 at 274 nm and stinging bee honey showed absorbance of 0.214 at 276 nm while the absorbance of sugar is 0.307 at 278 nm which is supported by the analysis using UV-Vis to represent sucrose. From here, both honey samples contain less amount of sucrose than the amount of sucrose present in sugar.

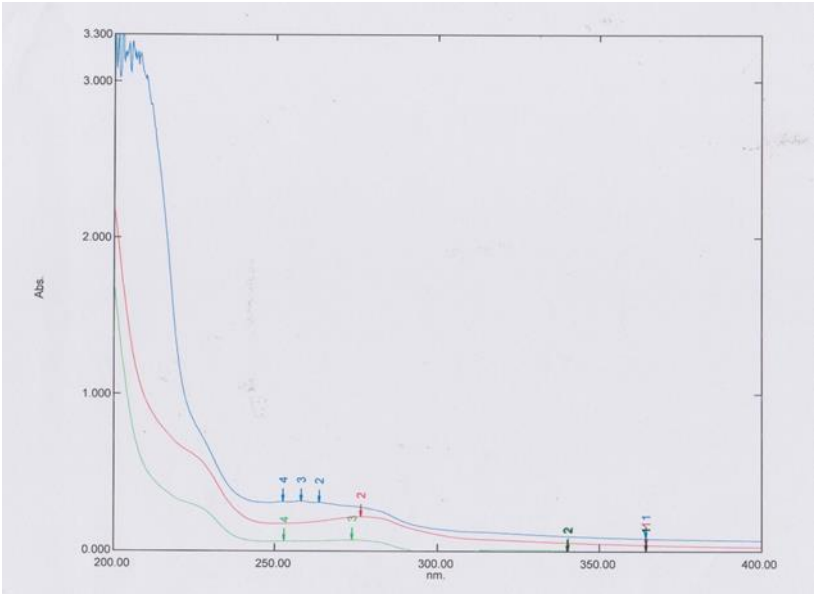

Figure 4. UV-Vis spectrum of (a) standard sugar (b) pure stinging bee honey and (c) pure stingless bee honey

3.3. Analysis of pure stingless and pure stinging bee honey using NMR spectroscopy

For ^{1}H NMR of pure stingless bee honey, pure stinging bee honey and sugar component was expected to show the chemical shifts δ in ppm. Figure 5 shows the NMR spectrum of sugar for ^{1}H profiling with D_2O solvent. From this study, the chemical shift at 4.70 ppm refers to the solvent peak which is D_2O. Meanwhile, chemical shift between 4.2 ppm and 3.0 ppm indicated to signals of the major region of sugar. The chemical shift for sugar showed glucose quantification at 3.25 ppm for β-glucose while 5.20 ppm indicated for α-glucose. There is a chemical shift at 4.19 ppm indicating presence of sucrose.

Table 1. 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

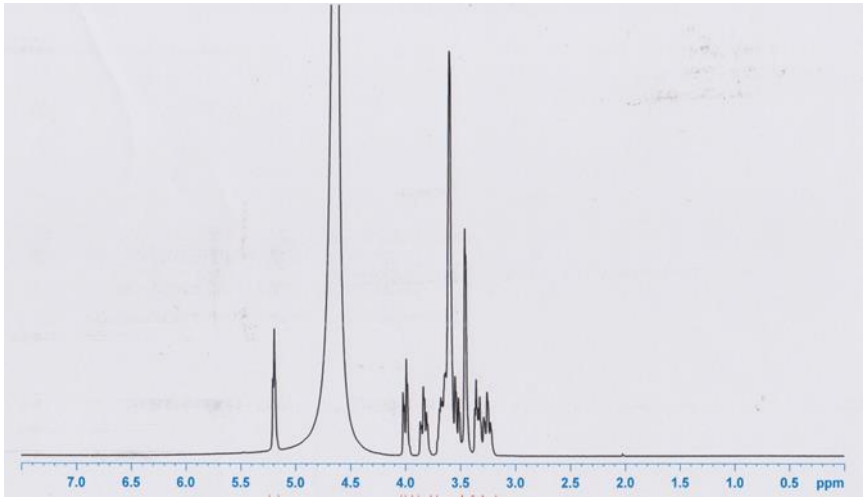

Figure 5. NMR spectrum of sugar

Figure 6 shows the NMR spectrum of pure stingless bee honey for ^{1}H profiling with D_2O solvent. From this study, the chemical shift at 4.67 ppm refers to the solvent peak which is D_2O solvent. There is no peak indicating α-glucose in the spectrum while at 3.25 ppm, there is presence of β-glucose. The chemical shift at 4.10 ppm and 4.42 ppm shows there are presence of fructose and sucrose in the honey.

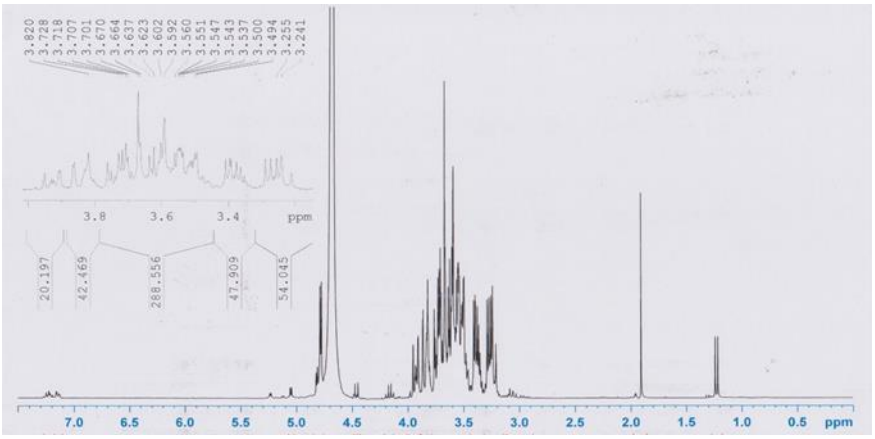

Figure 6. NMR spectrum of pure stingless bee honey

Figure 7 shows the NMR spectrum of pure stinging bee honey for ^{1}H profiling with D_2O solvent. From this study, the chemical shift at 4.60 ppm refers to the solvent peak which is D_2O solvent. There is no peak indicating α-glucose in the spectrum while at 3.24 ppm, there is presence of β-glucose. No peak indicates presence of fructose in the honey but at peak 4.30 ppm, there is presence of sucrose.

Sucrose is made up of glucose and fructose. The analysis of NMR spectroscopy showed fructose is present in stingless bee honey at 4.1 ppm while absent in stinging bee honey. Sugar shows a strong signal at 5.2 ppm indicating presence of glucose while absent in both honey samples. The study shows that both honey samples contain less amount of sucrose compared to the standard white sugar which have been melted and stingless bee honey is more nutritious than ordinary honey in terms of sucrose content.

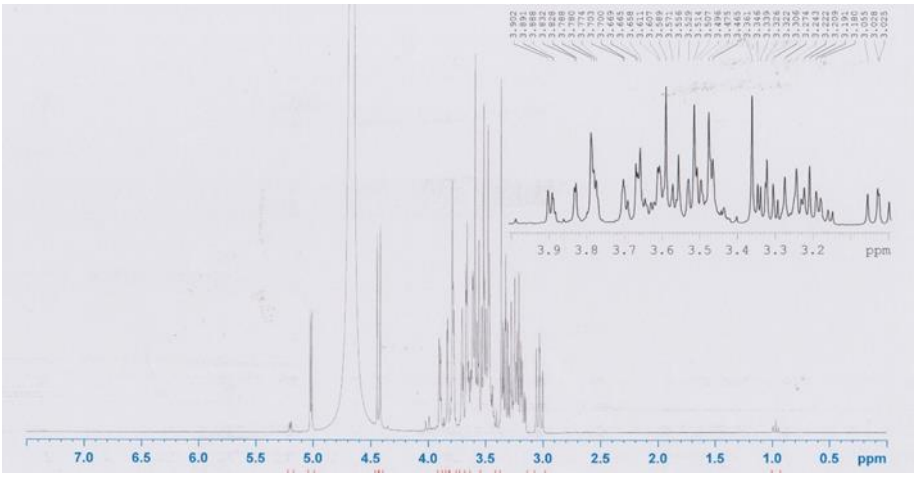

Figure 7. NMR spectrum of pure stinging bee honey

4. CONCLUSION

Through this study, three spectroscopic methods were used in the analysis of pure stingless bee honey, pure stinging bee honey and sugar. This analysis was done by using FTIR-ATR spectroscopy, UV-Vis Spectroscopy and NMR spectroscopy. These methods were simple and easy to be handled during the analysis. Moreover, these methods were not time consuming and the results obtained through these methods were reliable. As the conclusion, all these methods proved that pure stingless bee honey and pure stinging bee honey contain less amount of sucrose compared to the white sugar which have been melted and used as a standard. This has complied the Food Act where it is stated the percentage of sucrose in honey should be less than 10%. Apart from that, the pure stingless bee honey used is more nutritious than pure stinging bee honey. Stingless bee honey contains fructose which is the least cariogenic of the nutritive sugars.

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