Determination of Pure and Adulterated Stingless Bee Honey Based on Dielectric Properties

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Abstract
Recently, honey has become the target of adulteration due to high consumers demand and its nutritional value. This study was carried out to quantify adulteration of stingless bee (kelulut) honey from Heterotrigona Itama sp. based on its dielectric properties by using Precision Impedance Analyzer at frequency range of 40kHz to 40MHz. Five treatments of honey were prepared: 1) pure honey,2) adulterated with 15% water, 3) adulterated with 30% water, 4) adulterated with 15% sucrose and 5) adulterated with 30% sucrose. Result shows that dielectric constant (ε ') decrease as frequency increase and the best frequency to distinguished between pure and adulterated honey is at the range of 10MHz to 40MHz. It was found that as percentage of sucrose added in pure honey increased, the dielectric constant is decreased while as the percentage of water added in pure honey increased, the dielectric constant is increased. Strong negative relationship was found between dielectric constant with viscosity (R^2= 0.94), moisture content (R^2= 0.98) and soluble solid content (R^2= 0.81) at frequency of 40 MHz. From this result, it concludes that the dielectric properties of stingless bee honey can be used to differentiate between pure and adulterated honey and to determine viscosity, moisture content and soluble solid content of stingless bee honey.

Keywords: dielectric, stingless bee honey, adulterated, viscosity, moisture content, soluble solid content

Introduction

Stingless bee are also known as *Lebah Kelulut* in Malaysia with more than 38 stingless bee species have been identified but only four species are commercially cultivated: *Geniotrigona thoracica, Heterotrigona itama, Lepidotrigona terminata and Tetragonula leviceps* (Mustafa et al., 2018). Stingless bee honey has been claimed to have high medicinal beneficial than other bee species (Biswa et al., 2017). According to Souza et al. (2006), honey from stingless bee is more valuable and it has been used for a long time to treat various diseases. The recent studies showed that the stingless bee honey has the potential to treat colorectal cancer (Yazan et al., 2016), anti-inflammatory (Borsato et al., 2014), antimicrobial (Medeiros et al., 2016) and has an antioxidant property (Almeida Da Silva et al., 2013; Duarte et al., 2012). According to Chuttong et al. (2016b), stingless bee produce about 1–5 kg of honey per year depending on the species compared to *Apis mellifera* bee, with an average of 20 kg of honey per hive. Due to limited source and high demand of honey, the retail price of stingless bee honey is higher than common honey bee. Average stingless bee honey retail price is between RM250-RM300/kg (57 – 70 USD/kg). This has encouraged the flourishing of stingless bee honey industry in Malaysia. Nevertheless, with abundance of stingless bee honey product in the market, the purity of these honey is somehow questioned. According to the definition of the Codex Alimentarius and other international honey standards, honey shall not be added with others ingredient. However, natural honey had become targets of adulteration for economic gains due to its high demand due to its high nutritional value and the unique flavour characteristics There are several types of adulteration of honey in the industry, which are indirect adulteration of honey and direct adulteration of honey. Indirect adulteration of honey happens by feeding stingless bee with industrial sugar at bee farm. This type of adulteration is extremely hard to detect. Direct adulteration is the addition of foreign substances directly to honey for example adulteration of honey with sucrose syrup and water.

Recently, guaranteeing honey quality is getting to be progressively vital for consumers, producers and regulatory authorities. Therefore, detection of honey adulteration is very important. Various analytical techniques, including: isotopic (Padovan et al., 2003), chromatographic (Cordella et al., 2003a), and thermal analysis have been implemented for the detection of honey adulteration. The strength of these methods in honey adulteration detection has been proven by numerous researchers, however, they are time-consuming, destructive, and some of them are expensive. Therefore, fast, non-destructive, and precise analytical methods are encouraged to complement the existing technique. One of possible method is by using dielectric spectroscopy. Dielectric properties determine the interaction of electromagnetic energy with materials. It represented by a complex number, the relative complex permittivity, Ɛº = Ɛ' -jƐ" where the real part Ɛ' (dielectric constant) is associated with the capability of energy storage in the material, and the imaginary part Ɛ "(loss factor) associated with energy dissipation in the material in the form of heat (Guo et al., 2010). Extensive work has been done on a huge range of agricultural products and food which shows that the electromagnetic wave frequency and food compositions, particularly moisture content are the most vital factor influencing dielectric properties. In honey adulterations process, water is one of the common adulteration ingredients. Besides water, adulteration using additive sugars such as sucrose and fructose is also common and will change the composition of water content in the honey. Since water is the most important ingredient affecting the dielectric properties of a material, this has become the motivation to study the potential of dielectric properties to determine adulteration of stingless bee honey. Puranik *et al.* (1991) found that the addition of water to honey caused decreasing in relaxation time and increasing water content leads to the decreasing relaxation time. However, the correlations between the dielectric properties with other honey quality properties such as moisture content, soluble solid contents and viscosity is not yet discussed.

Materials and methods

Pure stingless bee honey from *Heterotrigona Itama sp.* was obtained at Ladang 10, Universiti Putra Malaysia (UPM). The pure samples have been harvested directly from the stingless bee hives that have been rear at farm to ensure only pure honey samples are used for this study.

1)Preparation of sample

There are five group of treatments for this study which are 1) pure honey, 2) adulterated with 15% water, 3) adulterated with 30% water, 4) adulterated with 15% sucrose and 5) adulterated with 30% sucrose (Figure 1). 5 samples with 50ml of pure honey were prepared for each group. Adulterated groups mixture was left in water bath at 45° C for about 3 hours to ensure that the sucrose and water added to the honey mixed well and to dissolve the presence of bubbles and crystal in the solution (Yanniotis *et al.,* 2006).

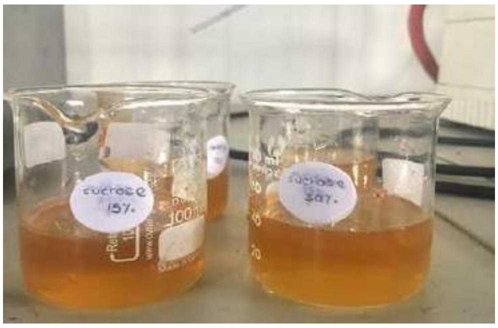

Figure 1: The honey sample inside viscometer cup

2) Dielectric properties measurement:

Dielectric properties measurement of each sample was performed by using a liquid test fixture Agilent 16452A, Agilent Technologies, Hyogo, Japan that connected to a Precision Impedance Analyzer Agilent 4294A, Agilent Technologies, Hyogo, Japan (Figure 2). Firstly, the dielectric test fixture was assembled and the shorting plate was inserted for fixture compensation. A 1.3mm spacer was used for the experiment. After the fixture compensation, the air capacitance (C_o) of the test fixture was measured at a room temperature of 25ºC. Five milliliters of stingless bee honey were poured in the inlet of the test fixture gently to avoid creating any air bubble. Then, the capacitance (C_p) and resistance (R_p) of the extracts were measured at frequency range between 40 kHz to 40 MHz. In between each sample measurements, the honey was drained, and the fixture was disassembled, cleaned and dried at room temperature. The test was replicated three times for each sample of extracts to determine the dielectric constant (Ɛ').

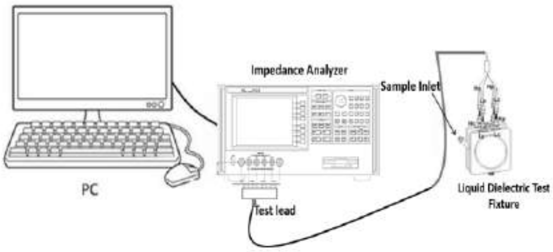

Figure 2: Dehydrator of stingless bee honey

3)Sucrose content and moisture content measurement

The sucrose content of all samples was measured using Digital Refractometer (D-22297, KRUeSS Optronic, Germany). Firstly, the switch button was turned on and one to two drops of sample was poured on the prism. Then, the sample prism was gently closed and the prism switch was rotate to lock it in place. The measurement knob was turn to set a boundary line to the intersection of the crosshairs while looking at eyepiece of the refractometer. The refractive index and temperature were displayed simultaneously on the LCD. The refractive index of honey is different from that of a sucrose solution at the same concentration, therefore the refractometer reading needs to be converted to percent moisture. Therefore, for honey, the water content is calculated from the refractive index measure, by applying the equation of Wedmore (1955).

$$W_{wed} = \frac{-0.2681 - \log\ (R.I - 1)}{0.002\ 243}$$

where

W_{wed} is the water content in g per 100 g honey; and

$R.I$ is the refractive index

Furthermore, to convert refractive index into brix reading, the table conversion according to 16th Session of ICUMSA 1974 was used. The brix reading was the value of soluble solid content or the sugar content of the samples. It defines the percentage by weight of sucrose in pure water solution.

4) Viscosity measurement

The viscosity testing was determined by using Sine-wave Vibro Viscometer (A&D SV-10, Tokyo,). It measures viscosity by detecting the driving electric current necessary to resonate the two sensor plates at constant frequency of 30Hz and amplitude of less than 1mm. A sample of 40 ml was poured into the cup until the surface reaches between the level gauges (Figure 3). Then the cup was attached to the table along the guides. Next, the protector was confirmed to in the position and the lever was raised to the sensor unit. Then, the knob was turned to adjust the sample surface to the center of sensor plate. Then, the viscometer was run to measure the viscosity of the samples. The viscosity reading was displayed on the unit of the viscometer in Pas or mPas.

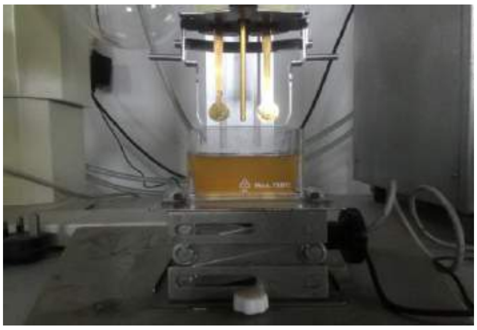

Figure 3: The honey sample inside viscometer cup

Results and discussion

Moisture content, soluble solid content and viscosity of pure and adulterated stingless bee honey

Moisture content, soluble solid content (SSC) and viscosity of pure and adulterated samples were measured and analyzed using statistical software SPSS. One way ANOVA with post-hoc Tukey HSD was used to determine difference between each group of treatment. Table 1 shows the average value of each honey properties with group of treatment.

Table 1: Average value for moisture content, soluble solid content (SSC) and viscosity of each treatment

Treatment	Average Moisture content (g/100g)	Average SSC (%)	Average Viscosity (pas)
Pure honey	26.76 ± 0.032 [a]	71.73 ± 0.029 [a]	0.38 ± 0.008 [a]
15% water	35.48 ± 0.427[b]	61.82 ± 0.845[b]	0.07 ± 0.004[b]
30% water	47.86 ± 0.516[c]	50.46 ± 0.585[c]	0.03 ± 0.001[c]
15% sucrose	26.25 ± 0.883 [a]	71.73 ± 0.357 [a]	0.42 ± 0.003[d]
30% sucrose	25.77 ± 0.563 [a]	72.6 ± 0.666 [a]	0.55 ± 0.001[e]

Table 1 shows that sucrose adulterated honey has higher viscosity compared to pure honey, while honey adulterated with water has lower value of viscosity. Honey adulterated with 30% of water have the highest moisture content value while honey adulterated with sucrose have slightly the same moisture content value with pure honey with no significant difference. This shows that moisture content increase with increasing water in honey. The result also shows that the SSC value has inverse relationship with moisture content. Honey adulterated with 30% of water have the lowest SSC value while honey adulterated with sucrose and pure honey have slightly the same SSC value with no significant difference using Tukey HSD mean comparison analysis. This shows that SSC value decreases with increasing water in honey and also proved that moisture content and soluble solid content analysis couldn't differentiate between pure honey and honey adulterated with 30% sucrose. The adulteration leads to reduced nutritional value of honey and could cause health concerns to the consumers with additive sugars. Honey adulterated with water has higher moisture contents which favours fermentation.

Variation of dielectric constant (ε') against frequency range of 40 kHz to 40 MHz

The dielectric constant for full sweep range frequency from 40 kHz to 40MHz is shown in Figure 4. From the graph, the pattern of dielectric constant for each group against frequency 40 kHz to 40MHz was difficult to differentiate and become constant as it reached to 10MHz. Furthermore, the slope between pure honey and adulterated honey is hard to distinguish since the line overlaps with each other. The value of dielectric constant at early frequency range from 40 kHz to 10MHz was not stable due to disturbances and noises

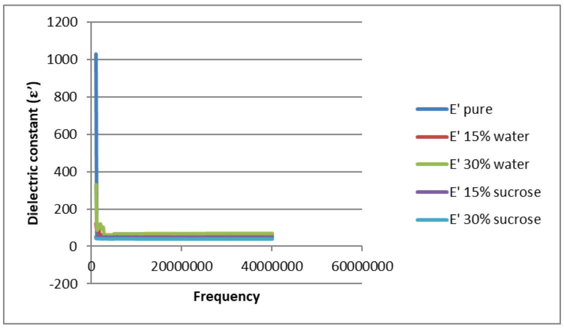

Figure 4: The dielectric properties of pure honey , honey adulterated with sucrose and water at frequency range from 40kHz to 40 MHz

Then, the range of frequency from 40 kHz to 40MHz was scale into two phase frequency range to form clearer pattern of dielectric constant for each group. The first range frequency analysed from 40 kHz to 10MHz and the second range frequency analysed from 10MHz to 40MHz. From Figure 5, the graph of first range frequency from 40 kHz to 10MHz is still difficult to analysed and quite similar with the graph of full range frequency. From Figure 6, the graph shows better pattern and dielectric constant for each group can be differentiate against the frequency of 10MHz to 40MHz. The pattern is clearer since less noise in between this frequency compared to the lower range frequency from 40kHz to 10MHz. This frequency range is then chosen to develop the regression graph and correlation.

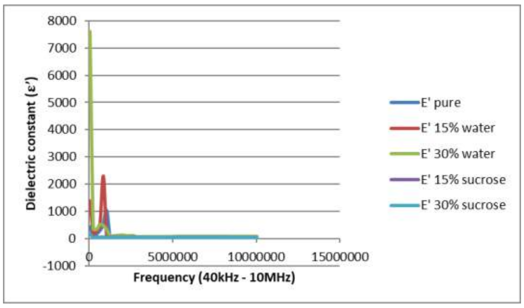

Figure 5: The dielectric properties of pure honey , honey adulterated with sucrose and water at frequency range from 40kHz to 10MHz

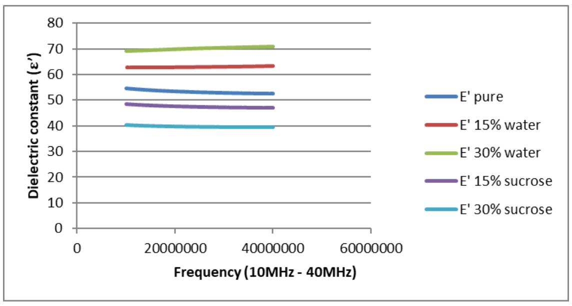

Figure 6: The dielectric properties of pure honey , honey adulterated with sucrose and water at frequency range from 10MHz to 40 MHz

The measured dielectric properties of five group treatments over the frequency range from 10MHz to 40MHz is shown in Figure 6. The result reveals that the dielectric constant of the five different treatments had the same frequency dependence. Honey adulterated with 30% water shows highest dielectric constant (70) compared to other treatment, and honey adulterated with 30% sucrose shows lowest dielectric constant (40). The graph also shows that pure (**ε**'=53***)*** and adulterated honey can be differentiate using dielectric constant value. Since dielectric constant has significant relationship with water content, it proved that honey adulterated with additional water has higher dielectric constant than others.

Relationship between dielectric constant (ε') with moisture content, SSC and viscosity.

The linear curve fit analysis was carried out on each quality parameters against dielectric constant model for each treatment in selected frequency value. All these frequencies were proceeded with regression analysis to generate the regression equation and selected the best linear regression model for quality parameter prediction. From the analysis, it was found that the best frequency that explained the relationship between dielectric constant and quality parameter is at 40MHz. Table 2 shows the regression equation for each quality parameter based on dielectric constant as functioning equation. It shows that each linear regression equation has high R^2.

Table 2: Regression equation for each parameter based on dielectric constant as functioning equation

Parameter	**Regression equation**	**R^2**
Viscosity	y = -0.018 **ε**' + 1.260	0.960
Moisture Content	y = 0.688 **ε**'-5.126	0.832
SSC	y = -0.699 **ε**'+ 103.85	0.844

Conclusion:

The dielectric properties on pure and adulterated honey has been measured between frequency of 40kHz to 40MHz using a liquid test fixture that connected to precision impedance analyser. From the results produce, dielectric properties of stingless bee honey shows promising result to be used as adulteration indicator and to develop a rapid, handheld sensor based on dielectric properties.

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