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Identification on Purity of Tualang and Kelulut Honey Through Measurement of pH and Electrical Conductivity

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ABSTRACT

There are abundant of replicated honey being sold in the market. Various tests and analysing techniques have been employed to verify the purity of honey, aiming to avoid consuming highly concentrated sugar solution that can cause various harmful effects on health. There is a growing interest on mobile authentication procedure to screen on purity of honey sample. In this study, screening for pure honey based on electrical conductivity and pH levels was analysed with the aim of simplifying screening process of isolating pure honey from replicated sample. The findings revealed that the electrical conductivity of pure tualang and kelulut honey ranged between 0.40 – 0.56 mS/cm and 0.80 – 0.93 mS/cm, respectively. All synthetic and adulterated samples were outside this range. As for pH levels, both tualang and kelulut were measured between pH 3.55 – 3.74 and 2.63 – 3.30, respectively, with several adulterated tualang and kelulut samples falling within the pH range of pure honey. Based on these findings, it is possible to isolate pure honey from replicated samples by measuring both electrical conductivity and pH, with electrical conductivity being particularly effective in distinguishing pure honey from both synthetic and adulterated samples.

1. Introduction

Honey is a sweet, viscous substance made by bees from flower nectar and honeydew. Bees collect honey and transform it through combination with specific substances of their own; then deposit, dehydrate, store, and leave it in honeycombs to ripen and mature. There are various types of honey are available which can be distinguished based on types of bees that produced the honey and sources of pollen and nectar to produce the honey [1]. In Malaysia, tualang and kelulut honey are types of honey that commonly consumed due to its nutritious and bioactive properties such as antibacteria and anti-inflammatory. Tualang honey is a multiflora honey produced by *Apis dorsata*, which is also known as the giant honey bee that collect pollen and nectar from various wild plants in Malaysia's rainforest jungle [2]. Kelulut, or stingless bee honey, is a multiflora honey produced by stingless bees from the genus of *Trigona*, which are small in size like the fly [3].

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Due to its high demand and cost to obtain pure honey, honey ranks as the third most commonly fabricated food worldwide, following milk and olive oil. Irresponsible actions to produce and sell replicated honey publicly have emerged affecting the beekeeping industry and consumer confidence. Warning to the public advising extra caution when purchasing honey from the market are continuously issued, especially in case where there are no labels or available information about the product's origin. Ongoing efforts are underway to raise awareness and consciousness about avoiding the consumption of replicated honey, which may contribute to harmful effects on health such as diabetes, obesity, and high blood pressure [4]. It is crucial for consumers to be aware and make informed choices when purchasing honey to safeguard both well-being and the integrity of the honey industry.

Replicated honey samples are either produced by entirely mixing additional substances such as refined sugar, corn starch and distilled water, known as synthetic honey or by adulterating pure honey with additional substances to increase volume and profit, known as adulterated honey [5]. There are various methods have been utilised to differentiate between pure and replicated honey, encompassing both do-it-yourself (DIY) tests and laboratory analysis. The DIY home test includes a variety of assessments such as the smell test, taste test, solubility test, water test, heat test, crystallisation test and microwave test. Meanwhile, in laboratory analytical testing, the purity of honey is determined based on physicochemical properties and the presence of compounds [6], minerals and proteins in honey using chromatographic techniques [7], spectroscopic techniques [8], stable carbon isotope ratio analysis [9], chemometric-integrated analytical techniques and biosensor [5].

Recently, there has been a growing interest in utilising the DIY home test to identify the purity of honey [10]. This is due to its advantages of being less complicated, providing rapid results, and being mobile. Although laboratory analysis offers scientifically proven procedures and reliable results for analytical purposes, it requires users to travel and submit samples to authorised laboratories, as well as follow several time-consuming procedures, including sample preparation, machine calibration, and media preparation. As a result, users tend to avoid laboratory analysis altogether and are willing to rely on the DIY home test, despite its lack of scientifically proven procedure. Therefore, independent tests to identify the purity of honey sample that offer accurate results, rapidity and high mobility are necessary.

The measurement of electrical conductivity (EC) and pH is common and can be performed anywhere. EC and pH serve as good criteria for determining the botanical origin and quality control of honey [11]. EC depends on the presence of organic acids, mineral, and ash of honey. The higher the presence of acids, minerals and ash, the higher the resulting conductivity. An EC of less than 0.8 mS/cm indicates blossom honey, while more than 0.8 mS/cm indicates honeydew honey [12]. The EC for tualang and kelulut honey recorded with range of 0.7 to 1.4 mS/cm and 0.5 to 8.8 mS/cm, respectively [13]. As for pH, it correlates with the content of organic acids such as gluconic acid, resulting from enzymatic action during the ripening of the nectar [14]. Tualang and kelulut honey commonly record a pH range of 3.6 to 4.0 and 2.4 to 4.2, respectively [13, 15, 16]. However, for both EC and pH, no specific range of standard acceptable limits is restricted to honey actively harvested in Asian countries, including Malaysia, despite the various types of honey being harvested, sold and consumed by the community in these countries, such as tualang and kelulut honey. Therefore, this project aims to identify EC and pH levels of tualang and kelulut honey and compare the values to the adulterated and synthetic honey samples. The goal is to assess the potential of isolating pure and replicated honey samples based on EC and pH values to possibly offer accurate, rapid and highly mobile authentication screening procedure.

2. Methodology
2.1 Collection and Identification of Pure Honey Samples

This study employed two types of Malaysian honey, namely kelulut and tualang honey. Total of twelve honey samples with six samples for each type of honey were obtained from different local apiarist and beekeeper around Malaysia, respectively. Upon collection, information regarding the collected honey was obtained through Certificate of Analysis (CoA) that have been accredited by the authorised institutions such as the Malaysian Agriculture Research and Development Institute (MARDI), Food Quality and Safety Research and Development (UNIPEQ), Kedah Bio-resource Corporation (KBioCorp), and Central Laboratory Universiti Malaysia Pahang (UMP) for its content. Due to variations in the content descriptions provided by different institutions for the same type of honey, an additional assay using the RapidRaw™ method was necessary to verify the honey's content. This test was conducted at the Malaysia Genome and Vaccine Institute (MGVI) laboratory following standard procedures described previously [17]. Out of twelve samples tested, ten were confirmed as raw honey based on the RapidRaw™ analyses. The ten honey samples which confirmed as raw honey were selected for further investigation. All selected samples were stored in sterile glass bottles, kept in the dark plastic container away from direct sunlight at room temperature within three to six months for data collection and appropriately labelled (as indicated in Table 1).

Table 1
Labels for pure honey samples

Types of Honey	Sample Number	Label
Tualang	1	T1
	2	T2
	3	T3
	4	T4
	5	T5
Kelulut	1	K1
	2	K2
	3	K3
	4	K4
	5	K5

2.2 Preparation of Adulterated and Synthetic Honey Samples

In this study, both synthetic and adulterated samples were included to compare the EC and pH levels between counterfeit and pure honey samples. For synthetic honey, two samples were prepared to replicate the chemical and physical properties of pure honey. The first synthetic honey sample (S1) was prepared by adding 33.5g of glucose, 40.5g of fructose, 1.5g of sucrose and 7.5g of maltose to distilled water to achieve a 100mL solution. This synthetic honey was prepared based solely on the standard sugar composition of honey [16,18]. The second synthetic honey sample (S2) was prepared using the same procedure, with the addition of 1g of citric acid to reach 100mL solution. The inclusion of citric acid was intended to mimic the acidic pH of honey [19] and was chosen due to its prevalence as the predominant organic acid in honey [20].

The adulterated honey was produced by mixing a sample of pure honey with the prepared synthetic honey in a 1:1 ratio as recommended by previous publication [5], to double the initial volume of pure honey without majorly affect its physicochemical properties. As for adulteration procedure, three samples of pure honey were chosen randomly from five honey samples that were previously confirmed as raw honey. Both tualang and kelulut honey were considered in production of adulterated honey sample. All adulterated and synthetic honey samples were stored in sterile glass bottles and labelled according to Table 2. The details of pure, adulterated and synthetic honey samples used in this study were tabulated in Table 3.

Table 2
Labels for adulterated and synthetic honey samples

Types of Honey		Sample Contents	Label
Adulterated	Tualang	T1 + S1	AT1
		T1 + S2	AT2
		T2 + S1	AT3
		T2 + S2	AT4
		T3 + S1	AT5
		T3 + S2	AT6
	Kelulut	K1 + S1	AK1
		K1 + S2	AK2
		K2 + S1	AK3
		K2 + S2	AK4
		K3 + S1	AK5
		K3 + S2	AK6
Synthetic		Fructose + glucose + maltose + sucrose	S1
		Fructose + glucose + maltose + sucrose + citric acid	S2

Table 3
The details of pure, synthetic adulterated honey samples used in this study

Sample		Bee Species	Classification	Colour
Pure honey	Tualang	*Apis dorsata*	Multiflora	Dark amber
	Kelulut	*Trigona sp.*	Multiflora	Dark amber
Adulterated	Tualang	Produced in laboratory		Dark amber
	Kelulut	Produced in laboratory		Dark amber
Synthetic		Produced in laboratory		Light amber

2.3 Measurement of Electrical Conductivity

The EC of the honey samples was measured at a sample concentration of 20% (w/v), in accordance with the recommended procedure [21,22]. The diluted honey was prepared by adding distilled water to 20g of pure honey to make up 100mL. The mixture was stirred for 10 minutes to ensure thorough mixing of the solution. After preparation, The EC value was measured in mS/cm using Hanna Instruments model HI98304® EC meter at room temperature of 25℃. The EC meter was calibrated before each use using calibration solution provided by the manufacturer. The measurements were carried out in triplicate to determine the average and standard deviation values.

2.4 Measurement of pH

The pH of the honey samples was measured using a 20% (w/v) solution. The test was conducted at room temperature of 25℃ with Hanna Instruments model HI98107® pH meter. The pH meter was calibrated before each use using calibration solution provided by the manufacturer. The average and standard deviation values were obtained from triplicate measurements.

3. Results

3.1 Electrical Conductivity of Pure, Synthetic and Adulterated Honey Samples

The EC of the samples was measured in mS/cm, and the results are presented in Figure 1(a) for tualang honey and Figure 1(b) for kelulut honey. For both figures, the bar chart illustrates the EC values for pure honey samples, while the reference lines indicate the EC values for synthetic and adulterated samples.

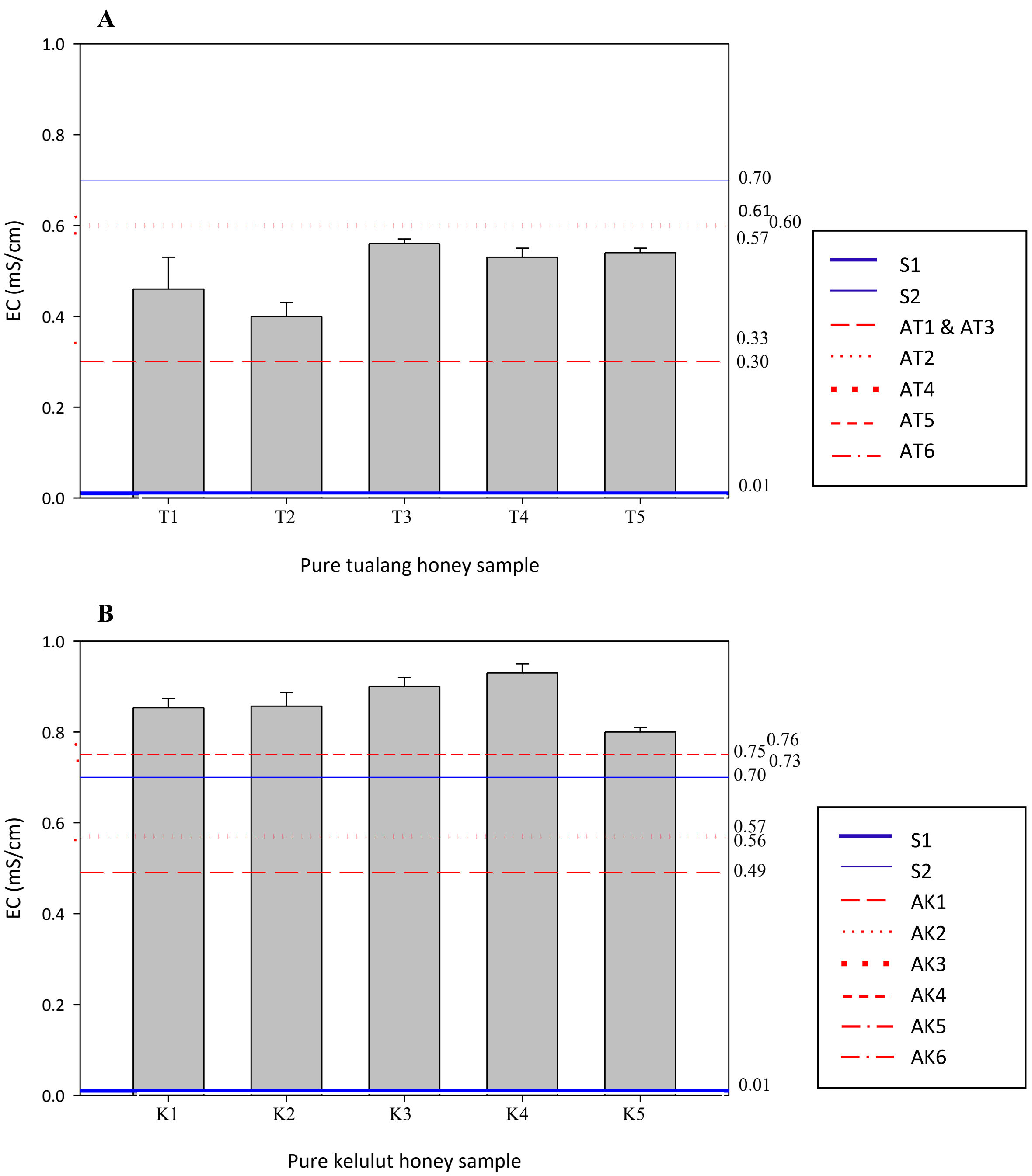

Fig. 1. The EC of pure, adulterated and synthetic honey samples for (A) tualang and (B) kelulut honey. Pure tualang honey samples (T1 – T5), pure kelulut honey samples (K1 – K5), adulterated tualang honey samples (AT1 – AT6), adulterated kelulut honey samples (AK1 – AK6), and synthetic honey samples (S1, S2)

The EC range for tualang honey samples, T1 to T5 was recorded between 0.40 ± 0.03 and 0.56 ± 0.01 mS/cm, with the lowest EC measured in samples T2 and the highest in sample T3. These EC levels were aligned with the European Honey Legislation and the Codex Alimentarius Standards, which stated that honey's EC should not exceed 0.8 mS/cm. However, some studies have reported EC values for tualang honey ranging from 0.30 to 1.13 mS/cm [23, 24], which is higher in range than the findings of current study. The variation in reported EC levels may be attributed to various factors in which the EC of honey is strongly associated with the content of minerals, nutrients, proteins, organic acids, ash and concentrations of ions [25], and honey with higher EC indicates high contents of these mentioned factors [26].

The EC of synthetic samples, S1 and S2, were recorded at 0.01 ± 0.03 and 0.70 ± 0.02 mS/cm, respectively, which were found to be outside the range compared to the EC of tualang honey samples, T1 to T5. The EC for S1 and S2 were differ by 41% and 98%, respectively, compared to the average EC value of tualang honey samples, T1 to T5. As for the adulterated samples, out of six samples, three samples (AT1, AT3, and AT5) were recorded at 0.30 ± 0.01, 0.30 ± 0.02 and 0.33 ± 0.02 mS/cm, respectively, which were 34% to 40% lower than the average EC value of tualang honey samples. However, the remaining three adulterated samples (AT2, AT4, and AT6) were recorded with higher EC values of 0.61 ± 0.01, 0.60 ± 0.01 and 0.58 ± 0.01 mS/cm, respectively. These EC values were 16% to 22% higher than the average EC value of tualang honey samples, T1 to T5.

Regarding kelulut honey, the EC for kelulut honey samples, K1 to K5, ranged from 0.80 ± 0.01 to 0.93 ± 0.02 mS/cm, with the lowest EC was measured in samples K5 and the highest in sample K4. These findings are consistent with other studies that reported the EC range for kelulut honey between 0.5 and 8.8 mS/cm [13]. When comparing kelulut and tualang honey, the EC values for kelulut honey were generally higher than tualang honey, being 43% higher on average. None of the kelulut honey samples recorded a lower EC than the tualang honey sample. The lower EC measured in tualang honey may indicate a lower amount of organic acids, minerals, and ash present in tualang honey samples compared to kelulut honey, which can be attributed to the smaller size of stingless bee that can collect pollen and nectar from various resources, unlike *Apis dorsata*, which is limited to larger floral sources for producing tualang honey [27–29]. Similar findings have been reported in other studies, showing lower EC values for tualang than kelulut honey [14, 15, 19]. When comparing kelulut and replicated samples, all synthetic and adulterated samples recorded lower EC values than kelulut honey samples, with values ranging from 12% to 99% lower compared to the average EC value for kelulut honey samples, K1 to K5. The addition of highly concentrated sugar into pure honey samples is expected to dilute the organic acids, minerals, and ash, resulting in lower EC values. The manipulation of adulterated honey samples by adding additional substances and excipients failed to mimic the EC level of pure honey. Similar findings have been reported regarding different EC levels for replicated samples compared to pure honey, resulting in either lower or higher EC values [30].

3.2 pH of Pure, Synthetic and Adulterated Honey Samples

The pH of the pure, synthetic and adulterated honey samples was measured at a concentration of 20% (w/v). The results are summarised in Figure 2, where the pH values for pure honey samples are depicted in bar charts, with reference lines indicating the pH values for synthetic and adulterated samples.

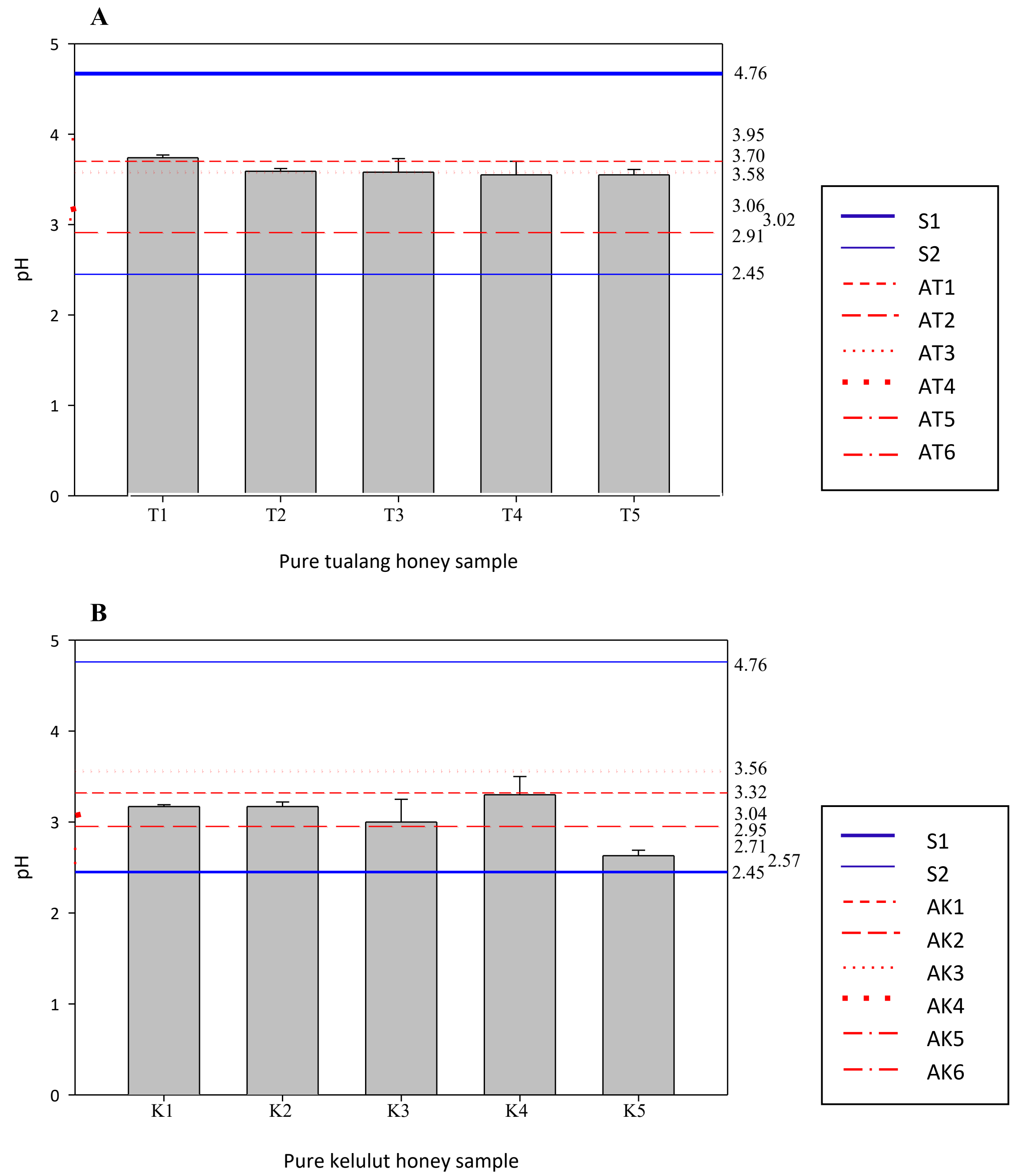

Fig. 2. The pH of pure, adulterated and synthetic honey samples for (A) tualang and (B) kelulut honey. Pure tualang honey samples (T1 – T5), pure kelulut honey samples (K1 – K5), adulterated tualang honey samples (AT1 – AT6), adulterated kelulut honey samples (AK1 – AK6), and synthetic honey samples (S1, S2).

The pH range for tualang honey samples, T1 to T5, ranged from 3.55 ± 0.15 to 3.74 ± 0.03, with the lowest pH recorded for samples T4 and T5, and the highest for sample T1. These findings align with other studies which reported pH values between 3.6 and 4.0 [31,32]. Regarding synthetic honey, samples S1 and S2 exhibited pH values of 4.76 ± 0.03 and 2.45 ± 0.02, respectively. The pH values were found to be outside the range compared to tualang honey, T1 to T5. S1 showed a higher pH by 32%, while S2 was lower by 31% compared to the average pH values for tualang honey samples. The pH values for adulterated honey samples varied, with some falling below, within and above the pH range of tualang honey samples. Lower pH values were recorded for samples AT2, AT4 and AT5, with pH values of 2.91 ± 0.02, 3.06 ± 0.02, and 3.02 ± 0.01, respectively. These pH values were lower by 15% to 19% compared to the average pH value for tualang honey samples, T1 to T5. Conversely, a higher pH was recorded only for adulterated sample AT6, with a pH of 3.95 ± 0.02, which was 10% higher than the average pH value for tualang honey samples. Additionally, two adulterated samples, AT1 and AT3, fell within the range of pH for tualang honey, with pH values of 3.70 ± 0.01 and 3.58 ± 0.02, respectively.

Regarding kelulut honey samples, K1 to K5, the pH ranged from 2.63 ± 0.06 to 3.30 ± 0.20, with the lowest pH observed in sample K5 and the highest in sample K4. These findings align with other studies reporting the pH range for kelulut honey between 2.4 and 4.2 [15,32,33]. When comparing kelulut and tualang honey, the pH values for kelulut were generally lower than tualang honey, with an observed 15% lower average value. None of kelulut honey sample recorded a higher pH value than the tualang honey sample. Similar findings of lower pH for kelulut compared to tualang honey have been reported in other studies [15,16,19]. The synthetic samples, S1 and S2, exhibited a similar pattern to that observed in tualang honey, with both S1 and S2 falling outside the range compared to the kelulut honey samples, K1 to K5 which differ by 7% to 81%. Regarding the adulterated samples, three out of six samples recorded pH values within the range of pH for pure kelulut honey samples. These samples were AK2, AK4, and AK5, with pH values of 2.95 ± 0.02, 3.04 ± 0.02, and 2.71 ± 0.02, respectively. The remaining adulterated samples, AK1, AK3, and AK6, were found to be outside the range compared to the pH for kelulut honey samples, with pH values of 3.32 ± 0.01, 3.56 ± 0.02, and 2.57 ± 0.01, respectively.

Based on the findings, it was observed that the pH values for pure honey could not be completely distinguished from replicated honey samples, as the pH for several adulterated samples fell within the pH range for pure honey. This observation was consistent for both tualang and kelulut honey. The acidity of honey is linked to the presence of organic acids and the fermentation of sugars, which are responsible for the antimicrobial properties [32,34] and unique physicochemical characteristics of honey [21,22,35]. In this study, the production of a synthetic honey sample with a highly concentrated sugar solution resulted in a moderately acidic pH of 4.76, which was distinguishable from the pH of pure honey. However, the addition of citric acid led to a significant decrease in the pH level of the synthetic sample, resulting in an extremely low pH of 2.45, which exceeded the pH level of pure honey. This finding is consistent with previous studies that reported an action of manipulating highly concentrated sugar solutions resulting in either extremely strong or weak acidic pH levels that were outside the pH level of pure honey [30]. Contrarily, detecting and isolating adulterated honey sample solely based on pH levels proved to be challenging. The presence of various natural organic acids such as phenolic acid, platycogenic acid, kauranoic acid, lucidenic, and acetic acids in tualang and kelulut honey allows for a wide range of pH values [19, 36], which could potentially be manipulated for profitable purposes by increasing volume without significantly altering its acidic pH. Similar findings were reported in previous study, which showed equivalent pH levels for pure and adulterated samples, ranging from pH 3.5 to 5.5 and pH 2.0 to 6.0, respectively [6].

4. Conclusions

The study successfully determine the EC and pH levels of tualang and kelulut honey harvested in Malaysia, and compared them with synthetic and adulterated honey samples produced in laboratory. The findings indicate that pure honey samples can potentially be distinguished from replicated samples based on both EC and pH measurements, with EC being particularly effective in isolating pure honey from synthetic and adulterated samples, showing percentage of differences ranging from 12% to 99%. However, the pH values were recorded to be within the same range for pure honey and several adulterated samples. As a recommendation, the method of identifying pure honey from EC and pH measurement should be compared with available screening method which approved by authorised institutions to analyse on its accuracy. Also, a higher number of samples for tualang and kelulut honey should be considered to ensure the reliability of the method. Additionally, the method of determining purity of honey through EC and pH levels could be investigated according to species of bee, nectar or pollen sources and other geographical factors, to further analyse and refine the method, with the aim of streamlining and simplifying the initial screening process for pure honey.

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