Original Article

Comparing the physiochemical properties of natural, synthetic and adulterated honeys

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Background: The fraudulent practice of selling adulterated honey is wide spreading because of strong economic incentives. Therefore, the objective of this study was to compare the physiochemical properties of natural and synthetic honeys.

Methods: Tualang honey was used as natural honey, and the synthetic honey was prepared in the laboratory, whereas a few market-purchased honeys was used for comparison. The tested properties included pH, colour, electrical conductivity, viscosity, moisture, total soluble solid, antioxidant capacity and hydroxymethylfurfural content which are considered as rapid tests and no advanced analytical tool is required.

Results: Of the tested parameters, electrical conductivity and rheology seemed to be more significant for detecting honey adulteration. Adulteration with sugary substances could change the flow behavior of honeys. The significant difference could be seen from the viscosity profile of honeys at different shear rate after diluted to 50% w/v. Somehow, the antioxidant capacity which was expressed as radical scavenging activity and ferric reducing power was unable to detect adulteration in honey. This was because the high content of HMF in adulterated honeys also contributed to high antioxidant capacity.

Conclusions: The results indicated that no single method could differentiate natural and adulterated honey, the combination of the selected parameters, specifically conductivity, rheology and HMF was able to conform the quality of honey on the aspect of adulteration.

Keywords: Honey; adulteration; colour; viscosity; conductivity; hydroxymethylfurfural

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Introduction

Honey is a naturally viscous and sweet substance produced by honey bees from the nectar of flowers or secretions of living plants or excretions of plant-sucking insects on plants that the bees collect and transform with their own salivary enzymes, and then deposit in beehive to ripen and mature (1). It has been consumed as food and medicine by mankind since centuries. The demand for honey is getting increasingly higher, mainly due to the limited supply and remarkable benefits of honey consumption. The benefits are predominantly attributed to the therapeutic effects contributed by bioactive compounds in honey, in addition to its high nutritional level. Therefore, honey is susceptible to adulteration with cheaper sweeteners including syrups and

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molasses, and sometimes added with a cocktail of chemicals to produce desired colour and bubbles.

Many studies have been conducted to detect adulterated honey in recent years. Different advanced analytical techniques such as spectroscopy, chromatography, differential calorimetry, biosensor, and stable carbon isotope ratio mass spectrometry have been applied (2,3). Each of the methods has its advantages and limitations. The detection of fraudulent honey is mostly focused on the sugar profile. Honey adulteration with sugars has also directly altered the physicochemical and rheological properties of honey.

Gebremariam and Brhane (4) and Ribeiro *et al.* (5) observed the pH value of adulterated honey would increase if commercial sugars such as corn or cane syrups was added into honey. On the other hand, Oroian *et al.* (6) reported that the adulteration with hydrolyzed inulin syrup which is acidic in nature would decrease the pH value of honey. According to Naila *et al.* (7), adulterated honey appeared to be brighter, while pure honey would be more reddish. Anyhow, the colour of honey would also be affected during storage. The colour change is strongly dependent on the temperature of storage and moisture content in honey (8).

There were studies revealing that electrical conductivity was one of the reliable parameters to detect fraudulent honey (9). Honey conductivity is one of the characteristics monitored and recommended by the European Communities to be less than 0.8 mS/cm (10). The limit is applied if 20 g honey is diluted with 100 mL water before measurement. The electrical conductivity is the manifestation of ion movement which is strongly influenced by viscosity and temperature. The viscosity of honey was found to be in a broad range of 18,169 to 2,560 cP at 25 oC (11). Hydroxymethylfurfural is another critical parameter to monitor honey adulteration according to the European Communities. This parameter is also regarded as a quality indicator for honey freshness and thermally over-treated during processing. Previous data on hydroxymethylfurfural in honey reported by worldwide researchers had been collected and statistically analysed by Chua (12) who found that the HMF content was exponentially increased if honey was treated at high temperature (90–100 ℃), especially for long duration.

The objective of the present study was to compare the physiochemical properties of natural and synthetic honeys, since honey adulteration is getting serious nowadays. A few rapid assays such as pH, colour, electrical conductivity, viscosity, moisture, total soluble solid, antioxidant capacity and hydroxymethylfurfural content of honey samples were conducted on Tualang honey (natural honey) which is the popular honey in Malaysia, laboratory prepared synthetic honey and market purchased honeys for comparison. We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/lcm-20-30).

Methods

Honey samples

The natural Tualang honey was purchased from the Federal Agricultural Marketing Authority (Kuala Nerang, Kedah). Two market honeys which were claimed to be Tualang honey were sourced from the local market. One synthetic honey (100 g) was prepared by dissolving glucose (33.5 g), fructose (40.5 g), sucrose (1.5 g) and maltose (7.5 g) in 17 mL deionized water according to the method described by Cooper *et al.* (13). The synthetic honey was heated at 100 ℃ for 6 hours until its colour changed to the dark yellow. The loss of water was replaced for the subsequent experiments.

Determination of pH value

The pH of samples was determined according to the method described in the International Honey Commission (14). A digital pH meter (Mettler Toledo Delta 320, Shah Alam, Malaysia) was calibrated at room temperature using pH buffer 4 and 7. A 10 g sample was dissolved in 75 mL distilled water. The solution was well mixed before the reading was recorded.

Determination of electrical conductivity

The electrical conductivity was measured using the method described in the Europe Honey Commission (14). A well-mixed solution consisted of 20 g honey in 100 mL distilled water was measured using a conductivity meter (EcoScan COND 6+, Eutech Instruments, Singapore). The results are expressed in mS/cm.

Determination of colour intensity

Different concentrations of sample ranged from 20–50% w/v was prepared by dilution with distilled water. The sample solution was filtered with a 0.45 μm nylon filter prior to absorbance measurement using a spectrophotometer (UV1800, Shimadzu, Japan) at 450 and 720 nm (15). The

difference of absorbance at both wavelengths is expressed as colour intensity in mAU.

Determination of honey viscosity

The viscosity of samples (50% w/v) was measured at 30 and 40 ℃ using a Brookfield viscometer equipped with a spindle CP42 (model LV, MA, USA).

Determination of moisture content

A 2 g sample was placed on a disk and dried in oven at 105 ℃ for 16 hours (16). The weight of the sample was recorded after constant weight was achieved. The loss was due to the evaporation of water in sample.

Determination of total soluble solid

The prism of a digital pocket refractometer (Atago Pal-α, China) was cleaned with Kim Wipe. A drop of homogenized honey sample was placed on the prism for measurement. The total soluble solid was measured at 25 ℃ and the results were expressed in °Brix.

Determination of ferric reducing antioxidant power

The assay was carried out according to the procedures described by Benzie and Strain (17) who used the principle of reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to its ferrous, coloured form (Fe^{2+}-TPTZ) in the presence of antioxidants. The reagent contained 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3 M acetate buffer at pH 3.6. It was prepared daily and warmed to 37 ℃. A 200 µL sample (10 %w/v) was mixed with 1.8 mL of the reagent and the absorbance of the reaction mixture was measured spectrophotometrically at 593 nm after incubation at 37 ℃ for 10 min. The standard solutions of $FeSO_4 \cdot 7H_2O$ (100–1,000 µM) were used to construct a calibration curve. The results are expressed as µM Fe^{2+}.

Determination of radical scavenging activity

The assay was carried out according to the procedures described by Chua *et al.* (18) with minor modification. A 100 µL sample (100–600 mg/mL) was mixed with 1.9 mL DPPH (130 µM) in ethanol and 1 mL acetate buffer solution (100 mM, pH 5.5). The mixture was shaken vigorously and left for 90 min at room temperature in the dark place. The absorbance of the solution was measured at 517 nm. The radical scavenging activity was determined based on Eq. [1].

$$\text{Scavenging activity}(\%) = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100 \quad [1]$$

Determination of hydroxymethylfurfural by LC-MS/MS

About 5 g sample was weighed into a 15 mL test tube. The sample was diluted with 5 mL of 0.1% formic acid solution. The solution was stirred for 30 minutes. Then, it was transferred into a separating funnel and added in 10 mL chloroform for liquid-liquid extraction. The solution was left for 30 minutes to form a two layers solution after vigorously shaking. The lower layer of chloroform was transferred out and the remaining solution was washed again with another 10 mL chloroform. The step was repeated three times. The collected chloroform fractions were combined and dried until achieved constant weight. The dried white solid was dissolved in methanol for LC-MS/MS analysis.

A 5 µL sample was injected into an HPLC system (Dionex Corporation Ultimate 3000; Sunnyvale, CA) equipped with a C18 reversed phase XSelect HSS T3 column (2.1 × 100 mm, 2.5 µm) and a QTOF mass spectrometer (AB SCIEX QSTAR Elite; Foster City, CA). A binary gradient system consisted of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile) was used as mobile phase. The mass spectrometer was used to detect the presence of hydroxymethylfurfural using the fragments of m/z 127 (parent ion) and m/z 90 (daughter ion) in the positive ion mode. Nitrogen gas was used for nebulizing (40 psi) and curtain gas (25 psi). The voltage of ion spray was 5,500 V, the declustering potential was 40 V and the focusing potential was set at 250 V.

Results

Table 1 is the physiochemical properties of honey samples. The results show that all honey samples exhibited the acceptable range of pH (3.4–6.1) (19) and total soluble solid (≥75%) (20) in honey. However, synthetic honey was found to be higher pH values than the other honeys. The significant difference of Tualang honey compared to the other samples was its electrical conductivity and hydroxymethylfurfural content. The high electrical

Table 1 Physiochemical properties of honey samples

Sample	pH	Electrical conductivity (μS/cm)	Moisture (%)	Total soluble solid (°Brix)	FRAP (μM Fe^{2+}/100 g)	HMF (mg/L)
Tualang honey	3.69±0.12[a]	513.0±1.2[a]	18.2±1.1[a]	75.1±0.1[a]	790.88±21.12[a]	2.48±0.01[a]
Synthetic honey	4.49±0.23[b]	43.9±0.2[b]	15.0±0.8[b]	80.1±0.3[b]	865.87±32.1[a]	5.16±0.03[b]
Market honey-1	3.68±0.21[a]	131.0±1.9[b]	18.5±2.6[a]	73.6±0.8[a]	895.17±43.23[b]	18.15±0.44[b]
Market honey-2	3.11±0.71[a]	89.0±1.2[b]	9.6±2.1[b]	81.3±0.1[a]	980.95±12.69[b]	162.65±1.21[b]

The results are statistically significant with the P<0.05 if the superscript in the same column shares the different letter with Tualang honey.

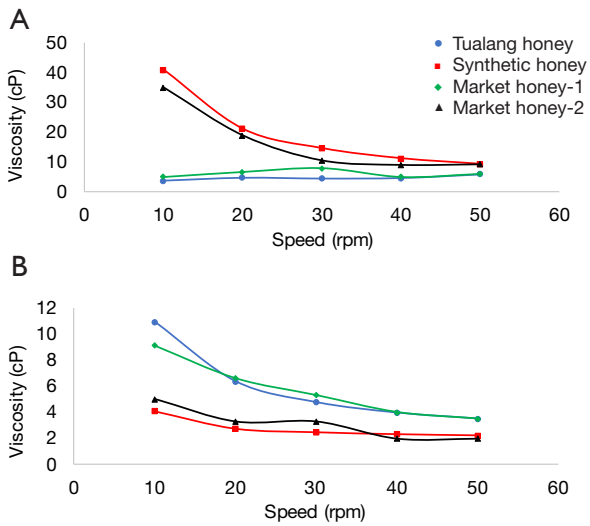

Figure 1 Viscosity of honeys diluted to 50% w/v at (A) 30 ℃ and (B) 40 ℃.

conductivity was attributed to the high mineral content of Tualang honey as published in previous studies (21). The synthetic honey and market honeys showed to have lower electrical conductivity which might be contributed by organic acids resulted from degradation of saccharides during preparation. The chemical degradation can also be seen from the high content of hydroxymethylfurfural in market honeys. Possibly, the market honeys were adulterated with sugar solution because its electrical conductivity was much lower than Tualang honey, but the value was just slightly higher than synthetic honey.

Honey is a viscous substance, usually behaves as Newtonian fluid. The viscosity of honey samples was measured at 30 and 40 ℃ in this study. The results found that all honey samples could achieve up to around 5,000 cP at 10 rpm with no significant difference among the samples. The honey samples also displayed Newtonian behavior in which the shear stress was linearly increased with the shear rate ranged from 19 to 190 s^{-1}. Similarly, the selected blossom and honeydew honeys from Czech Republic were also reported to be in Newtonian behavior (22). In 2015, researchers from India reported that adulterated honeys would display non-Newtonian behavior (23).

The honey samples were then diluted to 50% w/v and re-measured for their viscosity as presented in *Figure 1*. Dilution did not change the viscosity of synthetic honey and market honey-2 at 30 ℃ significantly. Both samples were also found to have the lower moisture content which directly contributed to higher viscosity. However, Tualang honey and market honey-1 was decreased to less than 1,000 cP at 10–50 rpm after dilution with water. The increase of temperature was further decreased the viscosity of Tualang honey and market honey-1 to less than 600 cP and less than 400 cP for synthetic honey and market honey-2. The observation noticed that a large change in the viscosity of synthetic honey compared to Tualng honey at 10 ℃ increment. The viscosity of synthetic honey was not affected by dilution at 30 ℃, but its value was significantly dropped to about 4 to 10 times lower at 40 ℃. On the other hand, the viscosity of Tualng honey was just decreased for about 0.5–3 times when the temperature was increased from 30–40 ℃. The viscosity of market honeys was in between the values of Tualang honey and synthetic honey. The flow behavior of market honey-1 was near to synthetic honey, whereas market honey-2 was close to Tualang honey.

The colour intensity of honey samples at different concentrations is presented in *Figure 2*. Synthetic honey and Tualang honey shows to have the highest and lowest colour intensity, respectively. Market honeys show to have comparable colour intensity from the concentration of 2–50% w/v.

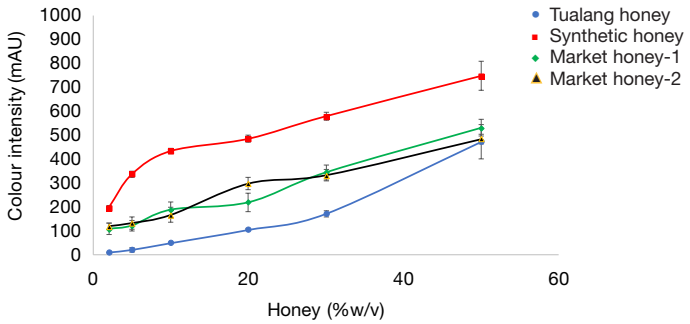

Figure 2 Colour intensity of honeys at the concentration ranged from 2–50% w/v.

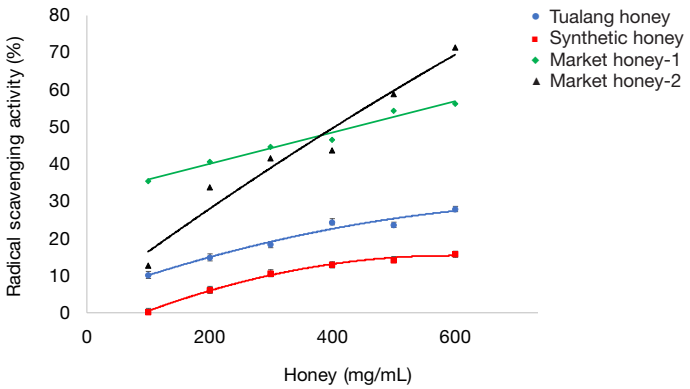

Figure 3 Radical scavenging activity of honeys at the concentration of 100–600 mg/mL

The DPPH assay showed to have an increment of radical scavenging activity when the concentration of honey samples was increased from 100–600 mg/mL (*Figure 3*). The figure shows that the scavenging activity of Tualang honey was slightly higher than synthetic honey, which was about 10% difference. However, the scavenging activity of both market honeys was much higher than Tualang honey and synthetic honey. This was in line with higher reducing power of market honeys. The high content of hydroxymethylfurfural might explain the high scavenging activity and reducing power of market honeys. Previously, Zhao *et al.* (24) reported that hydroxymethylfurfural could contribute to the antioxidant activity in a dose dependent manner. Therefore, the antioxidant capacity of honey samples could be contributed by the presence of botanical chemicals and hydroxymethylfurfural in honeys. Hydroxymethylfurfural has been used as a good indicator for honey adulterated with inverted sugars (25). However, this furan derivative could also be produced automatically in honey due to the degradation of reducing sugars in Maillard reaction, particularly after heating and prolonged storage.

Conclusions

Tualang honey was obviously different from synthetic honey in term of its electrical conductivity, colour, viscosity and hydroxymethylfurfural content. The hydroxymethylfurfural content was also contributed to the reducing power and radical scavenging activity of market honeys. Most probably, the market honeys were mixed with sugar solution and the mixing may involve thermal treatment which contributed to high hydroxymethylfurfural content. The electrical conductivity of market honeys was lower than Tualang honey, but higher than synthetic honey. To conclude, no single physiochemical test is capable to differentiate the natural honey and adulterated honey, especially the mixture of both honeys. However, the combination of the quick assays was able to conform the adulteration of honey satisfactory.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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