Correlations between Colour Lightness and the Sweetness of Stingless Bee Honey with Its Minerals Content

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ABSTRACT

The colour and the sweetness of honey are the main characteristics refers by consumers when buying honey for their consumption. Consumers always believe that the darker the colour and the sweeter the taste, the better the quality of honey. This study aims to investigate the correlation of colour lightness and the sweetness of stingless bee honey with its mineral contents. Honey samples were collected from seven beehives around Terengganu, Malaysia. All samples have different colours and tastes. The colour of honey was measured based on CIELAB colour system using a reflectance spectrometry and the sweetness of honey is measured using a refractometer. ICP-OES is used to analyze mineral elements present in the honey samples. We found that the lighter the colour of honey, the less sweet it is (r= -0.77). The results obtained also shows that the lighter the colour of honey, the higher the amount of potassium (r= -0.94) and phosphorus (r= -0.94) in honey. Meanwhile, the sweeter the honey, the higher the amount of potassium (r= 0.87), sodium (r= 0.85) and phosphorus (r= 0.82). Our findings prove that the colour and sweetness of honey can only be used to estimate the certain nutritive value of honey. Even though these findings still cannot fully uncover the myth of honey; consumers still can depend on these characteristics to choose the best honey for their own consumption.

Keywords: Stingless bee honey, honey colour, honey sweetness, honey quality

INTRODUCTION

Bees are herbivorous flying insects, obtaining all of their nutrition from the pollen and nectar of flowers. Bees gather and utilize nectar in order to produce honey, which provides essential nutrition for their survival. The biochemical composition of the nectar has a definite impression on the flavour, physicochemical and nutritional quality of honey (Rosidi Sujanto et al., 2021). There are two processes involves during the conversion of nectar into honey in the stingless beehive; (1) breaking down of sucrose into monosaccharide fructose and
glucose and (2) dehydration of extra moisture by consistently fanning the honeycomb with stingless bee wings. Bees secrete enzyme from their body and embed it into nectar to mature it into food, thus stop deterioration.

Honey is produced by both honey bee and stingless bee along which, both shown different compositions and properties (Kaškoniene & Venskutonis, 2010). Production of honey comes from various locations around the world, therefore, its quality in the global market is diverse. Classification of honey produced in Malaysia is either based on species or tribe of bee namely, *Apis* (stinging bee) for example; *A. dorsata*, *A. mellifera* and *A. cerana*, and Meliponine (stingless bee) for example; *Heterotrigona itama* and *Geniotrigona thoracica*. In addition, variations of Malaysian honey, for example, Tualang honey (Tualang Tree), Gelam honey (Gelam tree), Acacia honey (Acacia tree) and Rubber honey (Rubber tree) are contributed by various sources of available flora.

Bees visit a large number of flowers to collect a sufficient amount of nectars. Varieties of flowers and plants visited by these bees have great importance in determining honey's flavour and colour. The major variable to test on the honey quality, geographical changes, and seasonal changes as well as the floral source is based on its flavour and colour (Anupama et al., 2003). Honey is available in wide ranges of colour lightness (from almost colourless to a darker brown) and flavours (variation of mild and bold taste). The variations in both flavour and colour are influenced directly by the season of nectar (especially flower source), the interval between accumulating of nectar and harvesting of honey and also details in processing; heating, storage time and temperature (Ball, 2007; Terrab et al., 2004; Ramly et al., 2021). On the other hand, the colour and taste of honey are influenced by mineral content; highly concentrated minerals create darker colours and more potent flavour honey (González-Miret et al., 2005). Honey flavour and sweetness is correlated to the vaporizable compounds which range according to the seasonal conditions and geographical origin (Silvano et al., 2014). More than 75% and up to 95% of monosaccharides such as fructose and glucose are major constituents in honey (Bogdanov, 2016). Honey darkens over time. High temperature easily affects the colour of honey (cause colour darkening) and over some time, the honey easily deteriorate. Besides that, the colour of honey also gradually changes in cold temperature. Flavour and aroma deteriorate in a similar fashion when temperatures rise. Binnie (2018) stated that something as simple as prolonged warming in a bottling tank, a hot warehouse, or even a hot spot or shade in the kitchen has a degrading effect on honey.

Consumers have little knowledge in choosing a good quality honey. Most of the consumers claim that honey with darker-coloured is pure and more nutritious compared to the lighter-coloured, thus, the darker the most preferred. This claim has been known for so long and until now, it is still being used as a major preference. Other than that, consumers preferred sweet taste honey as it is considered delicious compared to others taste. The taste of stingless bee honey is varied; from sweet, sweet-sour and sour, depending on the source of the nectar collected. As the myths of honey are still untangled until now, the study was designed to investigate the correlation of the colour lightness and sweetness of stingless bee honey with its mineral contents. In addition, this investigation was done purposely to verify stingless bee honey quality and reveal the truth in reflects with sensory characters (specifically colour lightness and sweetness) of consumer preferences on honey in relation to its mineral content. This is able to confirm the infamous myth about honey in terms of its lightness and sweetness.

**MATERIALS AND METHODS**

**Honey Samples**

Seven samples of honey collected from four bee apiaries around Terengganu is used for analyses. Among these honey samples, four were produced by *Heterotrigona itama* (collected from Tembila, Benting Lintang, Kubang Jela and Banggol Peradong) while the other three were produced by *Geniotrigona thoracica*, *Lepidotrigona terminata* and *Heterotrigona erythrogastra* (collected from Banggol Peradong). The honey samples were collected by suction using the vacuum pump.
Colour

Colour parameters (L*, a*, b*) of the samples was quantified by using a reflectance spectrometry, Minolta Chroma Meter CR-400 colorimeter (Konica Minolta, Tokyo, Japan). The instrument was calibrated with a white background calibration plate prior to measuring the samples. The honey samples were put into Petri dishes on a white background, then, L*, a*, and b* values were measured based on CIELAB colour system. CIELAB colour system explains that the colour of honey become lighter upon the increasing value of colour lightness. Six replicates of each of the coordinates: L* for the lightness (black-white), a* for the redness (green-red) and b* for the yellowness (blue-yellow) were recorded.

![CIELAB colour system](image)

**Fig. 1. CIELAB colour system**

Total Soluble Solid

A refractometer (HANNA instruments-96801 refractometer digital) was used to measure the total soluble solid of honey samples. Approximately 0.3 ml of each honey sample was placed onto the prism surface of the refractometer. The values of total soluble solid in Brix were read in six replicates. Prior to measure the samples, the refractometer was zeroed with distilled water (Colucci et al., 2016).

Mineral Analysis

The approach of microwave digestion was implemented on all honey samples. Approximately, 0.5 g of each honey sample was added into 9 ml of 65% HNO3 and the mixture was digested in a microwave digestion system. The digested honey was diluted by marking up the solution up to 25 ml with ultrapure water. The same digestion protocol was applied on a blank. Approximately 10 ml of the sample was poured into a test tube for analysis using ICP-OES (Thermo Scientific iCAP 7000 Series, UK). The system of ICP-OES was set up so that the samples were analyzed three times for replication purposes. The obtained results from the instrument were expressed in mg kg⁻¹.

Data analysis

The data obtained were assessed by analyses in R statistical software version 4.0.3 (R Core Team, 2020). The correlation coefficient (R) between colour lightness and sweetness of stingless bee honey with its minerals content were subjected to Pearson’s correlation coefficient test. We conclude that the colour and sweetness (Brix) of honey are significantly correlated with the minerals content if the p-value of the test is less than the significance level alpha = 0.05. One-way ANOVA was used to determine the statistical significance of the result at P < 0.05.
RESULTS AND DISCUSSION

Colour Lightness versus Brix

Figure 2 shows the correlation coefficient of colour lightness with Brix. Accordingly, the higher the value of Brix, the lower the value of colour lightness, indicating high Brix value gives out the darker honey colour. The p-value of the test is 0.043, which is less than the significance level alpha = 0.05. It is concluded that colour lightness and Brix are significantly correlated with a correlation coefficient of -0.77. Chemical elements of plants from which the bee’s food sources originated, characterized the produced honey, therefore the presence of trace elements can be the indicator of the botanical origin of honey particularly (Celli & Maccagnani, 2003; Bogdanov, 2016). Research by González-Miret et al. (2005) found that dark coloured honey has higher trace elements contents than lighter colour ones.

Fig. 2. Graph of colour lightness against Brix
Fig. 3. Correlation between the colour lightness of honey with different types of minerals contained in the honey. The value of $p<0.05$ is significantly different between the two variables. (a): potassium, (b): sodium, (c): calcium, (d): magnesium, (e): manganese, (f): phosphorus, (g): iron, (h): copper, (i): zinc.
Fig. 4. Correlation between Brix of honey with different types of minerals contained in the honey. The value of $p<0.05$ is significantly different between the two variables. (a): potassium, (b): sodium, (c): calcium, (d): magnesium, (e): manganese, (f): phosphorus, (g): iron, (h): copper, (i): zinc.
Honey commonly contains a variety of minerals with each element varying in concentration even from the same botanical source, locality, geographical region, and same beehive even so in different greenery seasons (Atanassova et al., 2016). Minerals or trace elements identified and quantified in the stingless bee honey of the current study are potassium, sodium, calcium, magnesium, manganese, phosphorus, iron, copper and zinc as shown in Figure 3 and Figure 4. The minerals’ variation and quantity that are available in honey depend on the nutrient’s intensity in plants and soil accessibility, and contaminations of soil and environment. Potassium is the most abundant mineral in honey within the range of 3486.15 mg kg⁻¹ to 3688.35 mg kg⁻¹. This complies with previous studies on *Apis mellifera* honey which reported that the major mineral in honey is potassium (Popov-Ralić et al., 2015; Atanassova et al., 2016; Nayik & Nanda, 2016). The honey colour is part of the sensory characteristics with the centre of interest consequently determine its market price and its acceptability by the consumers. Tristimulus colorimeter that determines the colours of honey exhibited statistically and notably various values.

The correlation between the investigated variables of colour lightness and mineral contents is particularly conveyed in Figure 3. Potassium and phosphorus (both *r* = 0.94) were the only variables that show a strong correlation with colour lightness with *p*-value = 0.002 and 0.0018 respectively. Meanwhile, the other minerals do not show a statistically significant difference with colour lightness at least for the tested honey samples. Thus, colour lightness may play a part in the observed minerals intensity of the honey samples. Dark colour honey is in term with higher mineral concentration and conversely, light types are in term with lower mineral concentration. This pattern applied to all minerals not necessarily to potassium and phosphorus only. The previous study is in good agreement with this fact (Terrab et al., 2003; Vanhanen et al., 2011; Khalil et al., 2012; Moniruzzaman et al., 2013). González-Miret et al. (2005) categorized honey into two sets concerning their lightness value. It is considered light honey if the L* value is more than 50 and dark honey if L* value is equal or less than 50. The L* value of honey samples from the seven stingless bee honey types varied from 44.179 to 64.006. These values confirmed that the seven different sources of honey samples possessed both light and dark coloured honey. The correlation between colour lightness and minerals content of the samples suggests that potassium and phosphorus were the strongest factors that put up with the darkness of these honey than the rest of the minerals.

In general, honey produced by stingless bees brings about lower Brix values than honey produced by *A. mellifera* which is more than 75 °Bx. This is because stingless bee honey holds higher water content and conversely lower total sugars percentage. The soluble solids content (Brix) of the honey samples from this study extended from 67.72 to 71.69 °Bx. Data presented in Figure 4 revealed the correlation between Brix and nine minerals contained in the honey samples. A strong correlation exists and is detected between Brix with potassium, sodium and phosphorus (*r* = 0.87, 0.85 and 0.82 respectively) through which they exhibit statistically significant difference. These 3 mineral elements are major minerals that are present in the honey samples in the mean of exist in higher concentration than the other elements. Soluble solids content (Brix) value with the concentration of minerals is directly proportional to each other, and accordingly shows, high Brix value results in high mineral concentration. The Brix or total soluble solid is closely affiliated with the total sugars available in honey. Based on finding from Nayik & Nanda (2016), in honey itself, the sugar composition is determined by various factors such as the phytogeographic source of the honey, other than storage conditions and beekeeping practices. Biluca et al. (2016) affirm that the benchmark parameter of rate in solution solids (example: minerals) as soluble solids, at the same time directly connected to sugars amount and the water levels in the samples. It is an important criterion of having a higher Brix value for the determination of honey quality, especially during the market or storage period.

CONCLUSION

Our findings show the existence of a correlation between the colour lightness with the mineral contents in stingless bee honey. Moreover, a strong correlation is observed between colour lightness and each potassium and phosphorus with *p*-value= 0.002 and 0.0018 respectively, meanwhile, for the sweetness of honey, a strong correlation is recorded with each potassium, sodium and phosphorus with *p*-value= 0.011, 0.015 and 0.025 respectively. Being major minerals in honey, these elements of potassium, phosphorus and sodium does have a
strong correlation with the honey’s colour and sweetness which turn out to be darker and sweeter honey. In consequence, it is in agreement with sensory characters of consumer preferences thus verify that darker honey is denser in mineral and sugar content. Being mineral-dense, stingless bee honey is obviously a superior choice for human health when consumed.

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