Effects of Sugar Adulterants on the Physicochemical Properties of Natural Honey

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Abstract: Though honey is regarded as a safe and wholesome bee product, honey quality is very important to ensure it is safe for consumption and this effort still remained a challenge. The aims of this study are to determine and compare the physicochemical properties of natural and counterfeit honeys through experimental simulation. In this study, natural tualang honeys were compared with adulterated, synthetic and retail tualang samples from unknown sources. The physicochemical analysis observed include pH, free acidity, ash, total soluble solid, hydroxymethylfurfural (HMF) contents and electrical conductivity (EC). It was demonstrated that natural samples had lower pH and HMF but higher free acidity, ash content and EC. In contrast, adulterated samples showed decrease in free acidity, ash content, EC and increase in HMF. Apparently, synthetic samples had lowest free acidity, ash content and EC but higher HMF depending on type of sugar they contain. The same results as adulterated and synthetic samples were seen for retail tualang samples from unknown sources, suggesting altered quality of these products either due to adulteration or mimicry. Ash content was found useful to distinguish quality of honeys and may be considered as a reliable indicator of honey adulteration during processing.

Keywords: honey, sugar adulterant, quality, ash content, physicochemical analysis

Introduction

As a complex, supersaturated natural product of high value, honey medicinal values and health benefits were popular since ancient times. Honey mainly consists of carbohydrate and water, with other constituents such as minerals, vitamins, proteins, enzymes, organic acids, polyphenols, etc. are present in small quantities. Its composition is influenced by geographical distribution, floral sources, storage and processing [1]. Ironically, quality of marketed honey in some part of the world is unknown and increasing likelihood of honey being adulterated with various sugars and syrups to cope with limited production and increasing demand has shaken consumers' confidence. Potential adulterants include sugars and inexpensive sweeteners such as corn syrup (CS), high fructose corn syrup (HFCS), invert sugar (IS) and high fructose inulin syrup (HFIS) which can be added during beekeeping and/or processing [2-3]. While honey adulteration may not have deleterious short-term effect on human health, long-term consumption of high sugar adulterated honey such as those containing HFCS may lead to health problems including obesity and diabetes mellitus [4].

Several international guidelines provided by regulatory bodies such as European Union (EU), Food and Agriculture Organization (FAO) and International Honey Commission (IHC) are available to enhance quality control of honey. The Codex Alimentarius Commission [5] and European Union Directive [6] have outlined few parameters including moisture, sugar content, water insoluble solid, free acidity, diastase activity, hydroxymethylfurfural (HMF) and electrical conductivity (EC) as benchmarks of honey quality. In fact, sugar content and HMF are useful for identifying honey adulteration [7]. In Malaysia, the first findings of honey adulteration was reported by Yusoff *et al.* [8] who pointed out that 31 out of 40 honey samples of Malaysia origin tested for their sugars and HMF contents were found either adulterated or synthetic honeys.

Continuous efforts are made to characterize and differentiate quality of honey. Various single techniques have been employed to distinguish natural honey from adulterated and/or sugar solutions including fourier transform infrared spectroscopy (FTIR), near infrared spectroscopy (NIR), internal standard carbon isotope ratio analysis (ISCIRA), nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) [2, 9-12]. Besides, Subari *et al.* [13] used a hybrid sensing approach to characterize natural retail honeys from adulterated honeys where FTIR and e-nose fusion data were found more reliable and give better classification than single modality data.

No or limited study has been done to compare the physicochemical properties of natural and counterfeit honeys through experimental simulation. Differences in physicochemical properties of these samples were observed in this study.

2. Experimental section

2.1 Samples

Type and composition of honey samples are shown in Table 1. Samples N2 and N3 were positive controls while S2 and S3 were negative controls.

Table 1. Samples type and composition. [a]

Code	Honey type	Name (composition)
N1	Natural	Tualang (mixed source)
A1	Adulterated	75:25 (% v/v) N1:S1
A2	Adulterated	50:50 (% v/v) N1:S1
S1	Synthetic	Sugar syrup (243 g fructose, 201 g glucose, 9 g sucrose, 45 g maltose in 112 mL ultrapure water)
U1	Unknown	Tualang (unidentified)
U2	Unknown	Tualang (unidentified)
U3	Unknown	Tualang (unidentified)
N2	Natural	Tualang (mixed source)
N3	Natural	Tualang (mixed source)
S2	Synthetic	Sugar syrup (52 g fructose, 40 g glucose, 360.9 g cane sugar in 140 mL ultrapure water)
S3	Synthetic	Sugar syrup (HFCS, Invert sugar, etc.)

[a] HFCS = high fructose corn syrup.

2.2 Chemicals

Sodium hydroxide (NaOH), fructose, and HPLC grade methanol were obtained from Merck (Darmstadt, Germany). Glucose anhydrous was supplied by Fisher Scientific (USA). HMF and maltose monohydrate were procured from Sigma Chemical Co. Ltd. (St. Louis, Mo., USA). Sucrose was purchased from QREC (New Zealand). Cane sugar (sucrose) was procured from a local market.

2.3 pH and free acidity

pH of the samples was measured in a solution prepared with 10 g of sample in 75 mL of distilled water using a pH meter (Delta 320, Mettler Toledo, USA). Free acidity was determined by dissolving 10 g of samples in 75 mL of distilled water and titrated with 0.1 M NaOH to pH 8.3 [14].

2.4 Ash content and electrical conductivity (EC)

One gram of samples was ignited until completely dry and black [14]. The sample crucibles were then placed in a furnace (Thermolyne-Barnstead, USA) and incinerated at 600 °C for 6 hours. Ash content (g ash/100 g of honey) was calculated using the following formula:

$\text{Ash} = [(m_1 - m_2)/ m_0] \times 100$

where m_0 = sample weight, m_1 = weight of crucible + ash, and m_2 = weight of empty crucible

ECs of 20% w/v (dry weight basis) samples were determined using a conductivity meter (HI 98311, Hanna Instruments, Mauritius) in ultrapure water [14].

2.5 Total soluble solids (TSS) content

Total soluble solids (TSS) of 20% w/v samples were measured using a refractometer (E-line ATC 44-803, Bellingham-Stanley, UK). TSS measurements were further corrected for a standard temperature of 20 °C by including the correction factor 0.00023/°C [1].

2.6 HMF content

HMF content was determined using a HPLC according to methods described by the IHC [14]. Ten grams of sample was dissolved in 50 mL ultrapure water, filtered through 0.45 µm nylon membrane filter, and injected into an HPLC system (Agilent 1100, Agilent Technologies, USA) equipped with a photodiode array detector. The analytical column was a ZORBAX Eclipse XDB-C18 (4.6 x 150 mm, 5 µm; Agilent Technologies, USA). The mobile phase was 90 % methanol and 10 % water with a flow rate of 1.0 mL/min. The detection wavelength was set at 285 nm. The HMF concentration of each sample was calculated by comparing the corresponding peak areas of the sample and those of the standard HMF solutions after correcting for the honey dilution. There was a linear relationship ($R^2 = 0.9999$) between the concentration and the area of the HMF peak.

2.7 Statistical analysis

Data were presented as mean ± standard deviation (SD). The statistical differences of the measured parameters among all of the samples were analysed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test using SPSS version 16.0 program (IBM Corporation, New York, USA). A value of $P \leq 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1 pH and free acidity

Regardless of their type, all samples were acidic with pH values ranged from 3.05 - 4.02 (Table 2). However, pH values for synthetic samples were significantly higher than natural and adulterated samples. Adulteration of natural N1 honey resulted in a slight increase in pH as seen in A1 and A2 samples. pH highly influences the texture, stability and shelf-life of honey, therefore, is considered as an important factor during extraction and storage [15]. pH is also useful to indicate possible microbial growth especially for yeast and mould that can survive in acidic environments (pH = 4.0 - 4.5) [16]. The low pH as observed in natural samples (3.28 - 3.30) may represent the growth of some microbe in these samples.

Table 2. Physicochemical properties of analyzed samples (mean ± SD).[a,b]

Parameters	Samples							Positive Control		Negative Control	
(Units)	N1	A1	A2	S1	U1	U2	U3	N2	N3	S2	S3
pH	3.29 ± 0.01^{a}	3.34 ± 0.01^{b}	3.30 ± 0.01^{ab}	3.52 ± 0.03^{c}	3.05 ± 0.02^{d}	3.88 ± 0.01^{e}	3.28 ± 0.01^{a}	3.30 ± 0.02^{ab}	3.28 ± 0.02^{a}	3.94 ± 0.01^{f}	4.02 ± 0.00^{g}
Free acidity (meq/kg)	84.67 ± 0.42^{a}	80.70 ± 6.54^{a}	49.93 ± 1.10^{b}	14.73 ± 0.95^{c}	50.13 ± 0.50^{b}	5.20 ± 0.72^{d}	28.60 ± 0.20^{e}	70.00 ± 1.22^{f}	73.83 ± 0.98^{f}	3.67 ± 0.61^{d}	17.80 ± 1.51^{c}
EC (mS/cm)	0.84 ± 0.02^{a}	0.38 ± 0.02^{b}	0.51 ± 0.01^{c}	0.15 ± 0.02^{d}	0.29 ± 0.02^{e}	0.17 ± 0.02^{d}	0.24 ± 0.02^{f}	0.89 ± 0.02^{g}	0.75 ± 0.01^{h}	0.08 ± 0.01^{i}	0.25 ± 0.02^{ef}
Ash (g/100 g)	0.13 ± 0.01^{a}	0.06 ± 0.04^{bc}	0.07 ± 0.01^{b}	0.02 ± 0.02^{bc}	0.05 ± 0.01^{bc}	0.01 ± 0.00^{c}	0.02 ± 0.01^{bc}	0.16 ± 0.02^{a}	0.16 ± 0.01^{a}	0.01 ± 0.00^{c}	0.06 ± 0.03^{b}
TSS (°Brix)	75.67 ± 1.15^{ac}	73.78 ± 0.77^{a}	75.33 ± 0.34^{ac}	87.00 ± 1.73^{b}	77.89 ± 0.19^{c}	82.67 ± 0.58^{d}	82.34 ± 0.58^{d}	73.33 ± 1.53^{a}	74.33 ± 1.34^{a}	87.45 ± 1.68^{b}	63.00 ± 0.00^{e}
HMF (mg/kg)	46.54 ± 3.56	974.13 ± 23.01	842.46 ± 14.50	1132.60 ± 12.46	1051.50 ± 13.46	397.38 ± 1.76	537.45 ± 2.79	nd	nd	946.63 ± 12.30	84.45 ± 6.01

[a]N = natural; A = adulterated; S = synthetic; U = unknown; EC = electrical conductivity; TSS= total soluble solids; HMF = hydroxymethylfurfural; nd = not detected.

[b]Values are means ± standard deviation (SD) of three independent experiments except for HMF (two independent experiments). In each row, values with different letters (superscripts) are significantly differences ($P \leq 0.05$) by Tukey's post hoc test.

Free acidity of the samples ranged from 3.67 - 84.67 meq/kg (Table 2). Codex Alimentarius [5] specify free acidity should be less than 50 meq/kg indicating absence of undesirable fermentation. However, all natural samples showed higher free acidity (70.00 - 84.67 meq/kg). Free acidity of honey is attributed to the presence of organic acids, primarily gluconic acid, in equilibrium with their corresponding lactones or internal esters as well as inorganic ions such as phosphate, sulphate, and chloride [15]. Gluconic acid is produced when enzyme glucose oxidase in honey which normally secreted by bee as it deposits nectar and honeydew into the hive [17] converts glucose through oxidation process. In diluted honey, glucose oxidase is activated [18] and resulted in more gluconic acid. In the absence of glucose oxidase, no gluconic acid is produced and lower free acidity was expected as seen in synthetic S1-S3 samples (3.67 – 17.80 meq/kg) and unknown U2 sample (5.20 meq/kg). Also, higher free acidity in natural samples most likey linked to higher water content that aggravates honey fermentation by yeasts, producing acetic acid and a sour taste [1].

3.2 Ash content and EC

Ash content of the samples ranged from 0.01 - 0.16 g/100 g (Table 2). All natural samples showed significantly higher ash content (0.13 – 0.16 g/100 g) than other samples. Ash content measures the inorganic residue of honey after carbonization [19]. Codex standard specifies that mineral (ash) content for nectar honey should be ≤ 0.6 % and ≤ 1.2 % for honeydew honey [19]. With reference to the standard, it can be deduced that all natural samples are nectar honey (floral origin).

EC values of samples ranged from 0.08 to 0.89 mS/cm (Table 2). EC measures all ionizable organic and inorganic substances. Similar to ash content, EC value is relevant to botanical origin since this indicator can distinguish between nectar honey and honeydew honey. Nectar honey is defined as honey that is produced from the nectars of plants whereas honeydew honey comes from the excretions of plant sucking insects (*Hemiptera*) on the living parts of plants or secretions of the living parts of plants. EC values less than 0.8 mS/cm represent nectar honeys while honeydew honeys show EC greater than 0.8 mS/cm [5-6]. Nonetheless, natural samples exhibited higher EC values (0.75 – 0.89 mS/cm). Increase in conductivity of two natural N1 and N2 samples exceeded the limit for nectar honey could possibly be due to the influence of storage. A previous study demonstrated increased in EC (10–82 %) of sidder honeys stored for a year [20].

Both ash and EC are closely related to the mineral content of honey [21]. Lower ash and EC values for synthetic and adulterated samples may indicate less mineral content in these samples (Table 2). As for unknown samples, there is likely that these samples are either adulterated or synthetic due to the lower ash and EC values. Fig. 1 shows a linear relationship between ash content and EC of all samples in the present study. This finding is similar to earlier studies that reported this relationship for natural honeys from various floral sources and locations [19]. In addition, the same relationship was also observed for adulterated and synthetic samples as shown in Fig. 1.

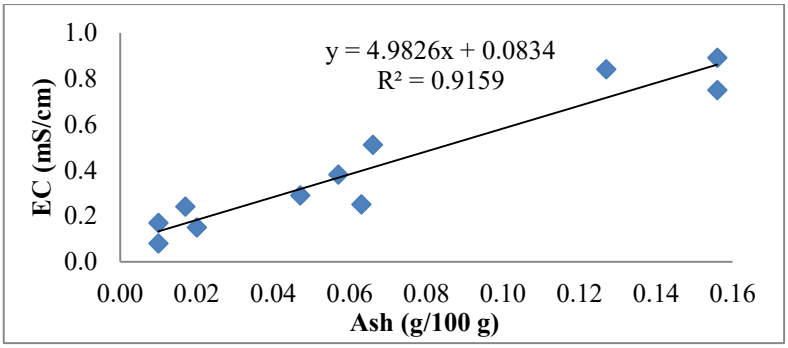

Fig. 1. Linear regression between ash content and electrical conductivity of all samples.

3.3 TSS

TSS, given in Brix degrees, may serve as an indicator of honey sweetness. The TSS of natural samples ranged from 73.33 - 75.67 ˚Brix (Table 2). Most of the total solids in the honeys are sugars with glucose and fructose as the main contributors [22]. Due to the presence of similar major sugar components but at varying concentration, TSS values between natural and adulterated samples were not much difference but the taste may differ. TSS values of natural samples were significantly different from synthetic and unknown samples.

3.4 HMF content

HMF content of samples ranged from 46.54 - 1132.60 mg/kg (Table 2). Natural samples N2 and N3 which were stored for less than 3 months showed undetected HMF level, thus confirming their freshness. However, natural sample N1 which has been stored for 7 months exhibited HMF value of 46.54 mg/kg, below the limit of 80 mg/kg set for honey from tropical countries [5].

As a parameter of honey freshness, HMF content is influenced by several factors such as prolonged heating and storage conditions (e.g. temperature). In honey, heating process converts sugars such as fructose and glucose into HMF from the acid-catalyzed dehydration of hexoses [1, 16]. Similarly, synthetic S1 and S2 samples which were prepared through prolonged heating exhibited higher HMF content (946.63 – 1132.60 mg/kg). Synthetic S3 most probably did not contain glucose and thus less affected. Surprisingly, unknown samples exhibited higher HMF content (397.38 – 1051.50 mg/kg) exceeded the limit of 80 mg/kg as outlined by the standard. This could be the results of prolonged heating to reduce water content and/or poor storage conditions. Adulterated samples also showed higher HMF (842.46 – 974.13 mg/kg) due to adulteration with prolonged heating sugar syrup S1 sample.

4. Conclusion

This study has shown that ash content is an ideal parameter that could be used to discriminate natural honey from adulterated and synthetic samples regardless of botanical and geographical origins, and storage duration. Although parameters such as EC and HMF could predict the presence of sugar adulterants in honey, these parameters are influenced by external factors such as storage conditions. Honey adulterated with sugar syrup was found to exhibit lower EC and ash content, but higher HMF. These study findings may be beneficial in regulating honey quality control and safeguarding consumer rights. Further studies need to be conducted on various types of honey and on a large number of samples for validation.

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5. References

[1] Saxena, S., Gautam, S., and Sharma, A. Physical, biochemical and antioxidant properties of some Indian honeys. *Food Chemistry*, Volume 118, (2010), pp. 391-397.

[2] Bertelli, D., Lolli, M., Papotti, G., Bortolotti, L., Serra, G., and Plessi, M. Detection of honey adulteration by sugar syrups using one-dimensional and two-dimensional high-resolution nuclear magnetic resonance. *Journal of Agricultural and Food Chemistry*, Volume 58, (2010), pp. 8495-8501.

[3] Ruiz-Matute, A.I., Rodríguez-Sánchez, S., Sanz, M.L., and Martínez-Castro, I. Detection of adulterations of honey with high fructose syrups from inulin by GC analysis. *Journal of Food Composition and Analysis*, Volume 23, (2010), pp. 273-276.

[4] Goran, M.I., Ulijaszek, S J., and Ventura, E.E. High fructose corn syrup and diabetes prevalence: a global perspective. *Global Public Health*, Volume 8, (2013), pp. 55-64.

[5] Codex Alimentarius. Revised standard for honey. Codex Standard 12-1981. Rev. 1 (1987), Rev. 2 (2001), Rome: FAO.

[6] European Union Directive. Council Directive 2001/110/EC relating to honey. (2002).

[7] Sanz, M.L., Del Castillo, M.D., Corzo, N., and Olano, A. 2-furoylmethyl amino acids and hydroxymethylfurfural as indicators of honey quality. *Journal of Agricultural and Food Chemistry*, Volume 51, (2003), pp. 4278-4283.

[8] Yusoff, K.M., Hamid, J,. and Manap, M.N.A. The presence of synthetic and adulterated honeys in Malaysia. Abstracts of the 1st International Conference on the Medicinal Uses of Honey (from Hive to Therapy). August 26–28, (2006). Kelantan, Malaysia. *Malaysian Journal of Medical Sciences*, pp. 123.

[9] Zhu, X., Li, S., Shan, Y., Zhang, Z., Li, G., Su, D., and Liu, F. Detection of adulterants such as sweeteners materials in honey using near-infrared spectroscopy and chemometrics. *Journal of Food Engineering*, Volume 101, (2010), pp. 92-97.

[10] Zakaria, A., Shakaff, A. Y. M., Masnan, M. J., Ahmad, M. N., Adom, A. H., Jaafar, M. N., Ghani, S. A., Abdullah, A. H., Aziz, A. H. A., Kamarudin, L. M., Subari, N. and Fikri, N. A. A biomimetic sensor

for the classification of honeys of different floral origin and the detection of adulteration. *Sensors*, Volume 11(12), (2011), pp. 7799–7822.

[11] Tosun, M. Detection of adulteration in honey samples added various sugar syrups with 13C/12C isotope ratio analysis method. *Food Chemistry*, Volume 138, (2013), pp. 1629–1632.

[12] Wang, S., Guo, Q., Wang, L., Lin, L., Shi, H., Cao, H. and Cao, B. Detection of honey adulteration with starch syrup by high performance liquid chromatography. *Food Chemistry*, Volume 172, (2015), pp. 669–674.

[13] Subari, N., Saleh, J.M., Shakaff, A.Y.M., and Zakaria, A. A hybrid sensing approach for pure and adulterated honey classification. *Sensors*, Volume 12, (2012), pp. 14022-14040.

[14] Bogdanov, S. Harmonised methods of the International Honey Commission. *International Honey Commission*, (2009), pp. 1-63.

[15] Terrab, A., D´ıez, M.J., and Heredia, F.J. Characterisation of Moroccan unifloral honeys by their physicochemical characteristics. *Food Chemistry*, Volume 79, (2002), pp. 373-379.

[16] Silva, L.R., Videira, R., Monteiro, A.P., Valentão, P., and Andrade, P.B. Honey from Luso region (Portugal): Physicochemical characteristics and mineral contents. *Microchemal Journal*, Volume 93, (2009), pp. 73-77.

[17] Cooper, R. Honey in wound care: antibacterial properties. *GMS Krankenhaushygiene Interdisziplinär*, Volume 2, (2007), pp. 1-3.

[18] Tan, H.T., Rahman, R.A., Gan, S.H., Halim, A.S., Hassan, S.A., Sulaiman, S.A., and Kirnpaul-Kaur, B.S. The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. *BMC Complementary and Alternative Medicine*, Volume 9, (2009), pp. 1-8.

[19] Feás X., Pires, J., Iglesias, A., and Estevinho, M.L. Characterization of artisanal honey produced on the Nortwest of Portugal by melissopalynological and physico-chemical data. *Food and Chemical Toxicology*, Volume 48, (2010), pp. 3462-3470.

[20] Qamer, S., Muzaffar, N., Ali, S.S., and Shakoori, A.R. Effects of storage on various honey quality parameters of unifloral sidder honey from Pakistan. *Pakistan Journal of Zoology*, 41, (2009), pp. 313-316.

[21] Chua, L.S., Abdul-Rahaman, N.-L., Sarmidi, M.R., and Aziz, R. Multi-elemental composition and physical properties of honey samples from Malaysia. *Food Chemistry*, Volume 135, (2012), pp. 880-887.

[22] Babarinde, G.O., Babarinde, S.A., Adegbola, D.O., and Ajayeoba, S.I. Effects of harvesting methods on physicochemical and microbial qualities of honey. *Journal of Food Science and Technology*, Volume 48, (2011), pp. 628-634.