Sugar profile and enzymatic analysis of stingless bee honey collected from local market in Malaysia

Sugar profile and enzymatic analysis of stingless bee honey collected from local market in Malaysia

W N Julika[1], A Ajit[1,4], N Ismail[1], N Aqilah[1], A Naila[2] and A Z Sulaiman[3]

[1] Faculty of Chemical and Process Engineering Technology, College of Engineering, Universiti Malaysia Pahang, 26300 Gambang, Kuantan, Pahang, Malaysia.
[2]The Maldives National University, Central Administration, Rehdebai Hingun, Machangolhi, Male, Maldives
[3]Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan, Kampus Jeli, Locked Bag 100, 17600 Jeli, Kelantan, Malaysia

Corresponding author: azilahajit@ump.edu.my

Abstract. Commercial honey is widely available in the market, raising questions whether the honeys are good in quality or otherwise, Thus, this research was designed to compare the quality of harvested stingless bee honey and commercial honey available in the Malaysian market by measuring their sugar profile and enzyme activity. The analysis showed that the honey contained moisture between 16.6% - 32.1%, various sugar starting with fructose (15.03 – 48.44 g /100 g), glucose (12.16 – 40.09 g/100 g), sucrose (<0.01 – 7.29 g/100 g), Fructose + Glucose (F+G) (15.03- 80.25 g/100 g), Fructose/Glucose (F/G) (0.78 – 1.63), and G/W (0.47 – 1.89). Also, diastase activity and Invertase activity of the honey varied from 1.82 to 6.11 DN and 0.27 IN to 4.94 IN, respectively. Eight honey samples including harvested honey, *H. Itama* and *G.Thoracica* showing comparable results with past studies and within the limits of Malaysian Standard. However, all honey samples demonstrate lower enzyme activity suggesting that honey from stingless bee has low enzyme activity compare to *Apis mellifera* honey.

1. Introduction

Recent years have seen growing interest among the consumers into food that helps maintaining their health with honey being one of them. Honey is a golden sticky liquid food that is sweet and rich in nutrients due to their various components such as sugars (mostly fructose and glucose), enzymes, amino acid, proteins, organic acids and mineral [1,2]. Stingless bees honey is not very popular to consume due to its sour and bitter taste [3]. Furthermore, stingless bee honey colour are darker, more watery in texture and expected to have slower crystallization compare to *Apis mellifera* honey [4,5]. Enzymes have always been an important subject in research involving honey as it can be used to differentiate between pure and adulterated honey. The predominant honey enzymes are diastase (amylase), invertase, glucose oxidase, as well as catalase and phosphatase [6]. However, this research is focussing mainly on diastase and invertase activity as both enzymes are largely used to measure honey quality freshness. Almost 95–99% of honey's dry substance consist of sugars where fructose is the most dominant followed by glucose and sucrose [7]. There is only small amount of sucrose (1% w/w) are found in honey due to the presence of invertase enzyme[8–10]. Furthermore, the quality of honey also is relying on the sum of fructose and glucose, fructose/glucose ratio and glucose/water ratio [11]. Due to the increasing popularity of honey, the honey supplied are decreasing thus it has led to the

production of impure honey. In a year, stingless bee only can produce up to 4 kg honey per colony compared to *Apis meliffera* honey which can produce 5 to 9 kg honey per colony [12]. Therefore, to cater the additional demand and to gain more economical profits, the irresponsible producers tend to add cheap chemical and artificial syrup such as cane sugar into pure honey. The abundance adulterated honey in the market could negatively impact the consumer trust and most importantly their health thus in order to find the difference between pure and adulterate honey, this present study is intended to measure the sugar profile and the enzyme activity of several stingless bee honey samples in Malaysia. Simultaneously, the properties of harvested and commercial stingless bee honey were compared and the interaction between sugar profile and enzyme activity in stingless bee honey were also observed.

2. Materials and Methods

2.1 Sample Collection and Preparation

17 samples of stingless bee's honey were collected from May 2017 to March 2018. Two pure stingless bee honey, *Heterotrigona Itama and Geniotrigona Thoracica* were harvested from Universiti Malaysia Pahang stingless bee farm and Aqif Kelulut Farm, Pekan, while other 15 samples were randomly obtained from local market around Malaysia. The honey obtained was stored in the dark at ambient temperature until the experiment.

2.2 Chemicals and Reagents

All of the chemicals and reagents used were of analytical grade. Sugar standard (Fructose, Glucose, Sucrose), acetonitrile, potassium hydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$), sodium chloride (NaCl), and hydrochloric acid (HCl) were purchased from Sigma-Aldrich (USA), and p-Nitrophenyl-α-D-glucopyranoside (pNPG) was purchased from Merck (Germany). Iodine was obtained from R&M (Malaysia) and tris- (hydroxymethyl) aminomethane was purchased from Nacalai Tesque (Japan).

2.3 Moisture content

Using the refractive index of the honey, the moisture content was measured by handheld refractometer (RHB 90ATC, China).

2.4 Sugar profile of Honey

Sugar profiles determination (fructose, glucose, sucrose) were performed using 1260 Infinity II LC System (Agilent Technologies, USA). The method is following the method of Malaysian Kelulut Standard [13]. The sugars were eluted through Phenomenex column (PhenoSphere 5µ NH_2 80A, 250 x 4.6 mm, Phenomenex Inc, USA) and detected by refractive Index detector (RID) operated at 35ºC. The mobile phase is acetonitrile: water (75:25, v/v) at a flow rate of 0.9 ml/min. The retention times obtained from the standards were compared to obtain HPLC sample peaks. The injections were performed in triplicate where the average peak area was used for evaluation.

2.5 Enzyme Activity

2.5.1 Diastase Activity

The diastase activity was determined by following the method of International Honey Commission [14]. A 5.0 g of honey samples were dissolved in 15 mL distilled water and then mixed with 2.5 mL of acetate buffer (1.59M, pH 5.3). The solution was then mixed with 1.5 mL of 0.5 M sodium chloride solution in 25 mL volumetric flask before 10 mL of this solution is taken and combined with 5 mL of 2% starch solution in a test tube. Then, the test tube was kept in BS -21 shaking water bath (Lab Companion, Jaio Tech Inc, South Korea) at 40ºC. After 5 minutes, 1 mL of the solution was mixed with 10 mL of 0.0007M diluted iodine solution. The absorbance was recorded using a spectrophotometer at 660 nm until the reading reached less than 0.235 absorbance. The diastase activity was expressed in Diastase Number (DN). DN was the amount of enzyme that hydrolysed/ converts 1% starch solution/ 0.01g of starch for 1 h at 40ºC.

2.5.2 Invertase Activity

The invertase activity was determined by following the method of International Honey Commission [14]. Substrate solution; p-Nitrophenyl-α-D-glucopyranoside (pNPG), was used in order for it to be dissolved into glucose and p-nitrophenol by enzyme invertase in honey [15]. The invertase activity of the samples was determined using UV- Vis spectrophotometer (ThermoFisher Scientific, USA) at 400 nm where the values obtained were expressed as IN (Invertase Number). The IN indicates the ability of enzymes to break down sucrose in 1 h [14].

2.6 Statistical Analysis

All analyses were prepared in triplicate where the differences between mean values were relevance at values of $p < 0.05$. The data obtained in the study were statistically analysed using analysis of variance (ANOVA) and followed by Tukey test (Minitab 18, Minitab Inc, USA).

3. Result and Discussion

3.1 Sugar profile of honey

The results of stingless bee sugar profile are presented in Table 1. The addition of fructose and glucose content of seventeen (17) honey samples were varied between 27.2 to 80.25 g/100 g with an average of 56.93 ± 16.5 g/100 g. The summation results met the requirement by Malaysian kelulut standard which is no more than 90.0 g/100 g. However, some samples slightly exceeded the codex standard of sugar profile for honey (< 60 g/100 g). For good quality honey, the glucose content should be lower than the fructose content [16] and this can be seen in most samples except for six commercial samples (C1, C4, C5, C10, C13 and C15). Thus, the six honeys probably were poor quality honey. Honey sample, C4, has high sucrose content of 7.29 ± 0.18 g/100 g, but it is still within the maximum limit stated by Malaysian kelulut standard which is no more than 8.0 g/100 g. High sucrose content could be contributed by various factors such as stingless bee species, floral sources, sucrose not completely converted into fructose and glucose and the trace of adulteration activity in honey [1,8,17].

3.2 Moisture Content

As shown in Table 1, moisture content varied from 16.6% to 32.1%, which is still in the range of Malaysian standard. For raw honey, the moisture should be no more than 35% and no more than 22% for processed honey. The results showed that, C11 had the lowest moisture content while C15 had the highest moisture content. Moisture is one of the significant characteristics in quality evaluation of honey as it control the maturation and preservation effects of honey, influences viscosity, weight, crystallization and finally the flavour of honey [18]. In addition, moisture content are able to prevent fermentation and granulation during storage [11]. Apart from being exposed to the fermentation process, higher moisture content can also indicate the honey was adulterated (> 35%) [10].

3.3 Ratio of Fructose/ Glucose (F/G) ratio and Glucose/ Water (G/W)

The F/G ratios influence the flavour of honey as the fructose is more sweet than glucose and sucrose [1,19]. Commercial honey, C11 has the highest F/G ratio of 1.90 (Table 1), thus the honey is sweeter compare to others. Besides that, F/G ratio implying the honey ability to granulate because when the amount of fructose is greater than glucose, the honey is in fluid state [20]. When the F/G ratio is below 1.0, the crystallization of honey is quicker, however when this ratio is greater than 1.0, the honey stays in liquid forms for a long time [11,21]. F/G ratios not only depend on the source of the nectar but it also depends on the variation of bee species and climate of different regions [4].Apart from F/G ratio, the G/W ratio also associated to honey crystallisation. Both ratios are useful to predict and control the chances of granulations in honey especially G/W ratio since the glucose contents and moisture content are crucial for honey granulation [11,16]. Honey with F/G more than 1.0 and G/W less than 1.0 are likely to crystallise slower compared to honey with low F/G (< 1.0) and high G/W (> 2.0) [19] . *G. Thoracica* has the lowest G/W value which is 0.46 (<1.0) thus, it may imply that the honey has the lowest ability to crystallize and will prolong as liquid for quite some time compared to other honey.

Table 1. Summary of sugar profile of various stingless bee honey samples from Malaysia (mean ± standard deviation, n = 3).

Samples	Fructose (g/100g)	Glucose (g/100g)	Sucrose (g/100g)	F+G (g/100g)	F/G	Moisture content (%)	G/ W
H. Itama	17.5 ± 1.3^{j}	16 ± 0.8^{j}	< 0.01	33.5 ± 2.27^{i}	1.10 ± 0.10$^{c, d, e}$	30.3 ± 0.18^{b}	0.53 ± 0.03^{i}
H. Thora	15.03 ± 1.22^{i}	12.17 ± 0.47^{k}	< 0.01	27.2 ± 0.69^{j}	1.24 ± 0.09$^{b, c, d}$	26.4 ± 0.09^{d}	0.46 ± 0.02^{i}
C1	30.96 ± 0.81^{e}	31.6 ± 0.79^{d}	1.10 ± 0.03$^{e, f}$	62.56 ± 1.53^{e}	0.98 ± 0.02^{f}	19.43 ± 0.20^{g}	1.63 ± 0.06^{b}
C2	32.52 ± 0.59$^{d, e}$	30.09 ± 0.70$^{d, e}$	< 0.01	62.62 ± 0.67$^{d., e}$	1.08 ± 0.04$^{c, d, e, f}$	28.43 ± 0.51^{c}	1.06 ± 0.02^{h}
C3	33.51 ± 0.54^{d}	31.94 ± 0.07^{d}	0.05 ± 0.02^{h}	65.45 ± 0.61^{d}	1.05 ± 0.01$^{c, d, e, f}$	26.7 ± 0.61^{d}	1.19 ± 0.03$^{f, g, h}$
C4	22.83 ± 0.42^{g}	25.47 ± 0.50^{g}	7.29 ± 0.18^{a}	48.3 ± 0.95^{g}	0.89 ± 0.01$^{d, e, f}$	17.73 ± 0.65^{h}	1.44 ± 0.08$^{c, d, e}$
C5	31.64 ± 0.48$^{d, e}$	34.55 ± 0.50^{c}	2.81 ± 0.09^{c}	66.19 ± 0.95$^{d, e}$	0.92 ± 0.01$^{d, e, f}$	23.43 ± 0.25^{e}	1.47 ± 0.01$^{b, c, d}$
C6	31.67 ± 0.57$^{d, e}$	28.08 ± 0.95$^{e, f}$	0.45 ± 0.13$^{g, h}$	59.75 ± 1.45$^{d, e}$	1.13 ± 0.02$^{c, d, e}$	26.7 ± 0.61^{d}	1.05 ± 0.01^{h}
C7	24.13 ± 1.80^{g}	22.83 ± 1.04^{h}	3.34 ± 0.33^{b}	46.97 ± 1.77^{g}	1.06 ± 0.02$^{c, d, e, f}$	17.12 ± 0.76^{h}	1.33 ± 0.11$^{d, e, f}$
C8	20.6 ± 1.10^{h}	18.53 ± 0.50^{i}	3.55 ± 0.05^{b}	39.13 ± 0.61^{h}	1.11 ±0.05$^{c, d, e}$	16.7 ± 0.35^{h}	1.11 ± 0.03$^{g, h}$
C9	42.81 ± 0.72^{b}	26.27 ± 0.63$^{f, g}$	3.35 ± 0.05^{b}	69.08 ± 1.35^{b}	1.63 ± 0.01$^{a, b}$	24.0 ± 1.0^{e}	1.89 ± 0.09$^{g, h}$
C10	39.35 ± 0.60^{c}	40.9 ± 1.03^{a}	0.80 ± 0.07$^{f, g}$	80.25 ± 1.62^{c}	0.96 ± 0.01$^{c, d, e, f}$	21.67 ± 0.57^{f}	1.89 ± 0.09^{a}
C11	48.44 ± 0.42^{a}	25.47 ± 0.50^{g}	< 0.01	73.91 ± 1.01^{a}	1.90 ± 0.02^{a}	16.6 ± 0.53^{h}	1.54 ± 0.06$^{b, c}$
C12	17.58 ± 0.44^{i}	13.33 ± 0.67^{k}	0.03 ± 0.002^{h}	30.91 ± 0.87^{i}	1.32 ± 0.07$^{b, c}$	28.43 ± 0.51^{c}	0.47 ± 0.02^{i}
C13	38.91 ± 0.30^{c}	37.06 ± 0.97^{b}	< 0.01	75.96 ± 1.21^{c}	1.05 ± 0.02$^{c, d, e, f}$	26.73 ± 0.55^{d}	1.38 ± 0.01$^{c, d, e}$
C14	26.05 ± 0.60^{f}	28.9 ± 0.85^{e}	2.17 ± 0.15^{d}	54.95 ± 0.86^{f}	0.90 ± 0.04$^{d, e, f}$	26.33 ± 0.32^{d}	1.09 ± 0.02$^{g, h}$
C15	31.04 ± 1.0^{e}	40.03 ± 1.0^{a}	1.47 ± 0.28^{e}	71.07 ± 1.01^{e}	0.78 ± 0.04$^{e, f}$	32.01 ± 0.30^{a}	1.25 ± 0.04$^{e, f, g}$
Mean ± SD	29.68 ± 9.42	27.25 ± 8.67	2.20 ± 2.06	56.93 ± 16.5	1.12 ± 0.28	24.04 ± 4.98	1.22 ± 0.43

Note: $^{a\ -\ k}$ =Means with different superscript letter along the column are significantly difference ($p<0.05$); F+G: Summation of fructose and glucose; F/G: Ratio of fructose to glucose; G/W: Ratio of glucose to water (moisture content)

3.4 Diastase Activity

Almost all honey contains diastase where the activity can be measured and expressed as Diastase Number (DN) [22]. Diastase activity for seventeen (17) samples of stingless bee honey ranged from 1.82 to 6.11 with average value of 3.89 ± 1.56 (Table 2). According to Codex Alimetarius [23], Diastase Number should be no less than 8 DN and for honey with low enzyme content, more 3 DN is acceptable. There is no fixed limit for diastase number in Malaysian kelulut standard. From the results, we can conclude that stingless bee honey has low diastase number compared to *Apis mellifera* honey since all samples are less than the limits for international standards. Our results also comparable with the study from Thailand [23] where the average diastase reported ranging from 0.050–4.9 DN. Diastase are regarded as one of quality criteria by international standard [24] where the values is influenced by honey storage and heating [25]. Besides that, it also can be used as a mark for honey freshness and overheating [26]. Diastase values are not only affected by geographical and botanical origin but also by pH values, nectar flow (honey flow) and foraging behaviour of the bees [27,28].

3.5 Invertase Activity

Invertase is the enzyme that hydrolyses sucrose to fructose and glucose. The invertase activity can be represented by either invertase units (IU kg-1) or as invertase number (IN), where 1 IN is equal to 7.344732 IU kg^{-1} [29,30]. Table 2 shows the invertase activity result for all samples. The invertase activity ranges were between 0.27 IN to 4.94 IN with average value of 2.77 ± 1.22 IN. No limits were proposed by Malaysian kelulut standard but according to Bogdanov [25], it was suggested that honeys should have more than 10 IN while for low enzyme honey, the activity should be greater than 4 IN. The fact that harvested honey, *H. Itama* and *G.Thoracica* had almost none invertase activity may suggest that the stingless bee honey in Malaysia has low enzyme activity compare to *A. mellifera* honey. Due to the low enzyme content, this may be regarded as a natural feature of these honey, rather

than an index of scarce freshness or lowered in quality. Both diastase and invertase activities steadily deteriorate on prolonged storage and heating of honey [31]. Nevertheless, invertase is considered as better freshness indicator than diastase because it is more susceptible towards prolonged storage and heat [25].

Table 2. Summary of predominant enzyme activity of various stingless bee honey samples from Malaysia (mean ± standard deviation, n = 3).

Samples	Diastase Number (DN)	Invertase Number (IN)
H. Itama	5.85 ± 0.08^{a}	ND
H. Thoracica	5.87 ± 0.48^{a}	ND
C1	$3.11 \pm 0.41^{f, g}$	3.2 ± 0.18^{d}
C2	6.11 ± 1.11^{a}	ND
C3	$3.79 \pm 0.01^{d, e, f}$	4.29 ± 0.41^{b}
C4	2.11 ± 0.20^{h}	0.27 ± 0.01^{g}
C5	$2.79 \pm 0.01^{f, g, h}$	$2.19 \pm 0.07^{e, f}$
C6	$5.36 \pm 0.25^{a, b}$	4.25 ± 0.13^{b}
C7	$4.33 \pm 0.17^{c, d, e}$	2.13 ± 0.07^{f}
C8	$3.33 \pm 0.17^{e, f}$	0.55 ± 0.05^{g}
C9	$2.16 \pm 0.13^{g, h}$	1.82 ± 0.07^{g}
C10	1.82 ± 0.16^{a}	3.79 ± 0.03^{c}
C11	$4.58 \pm 0.02^{b, c, d}$	ND
C12	5.65 ± 0.37^{a}	4.94 ± 0.12^{a}
C13	$5.12 \pm 0.11^{a, b, c}$	ND
C14	$2.32 \pm 0.12^{g, h}$	2.58 ± 0.09^{e}
C15	1.93 ± 0.06^{h}	$2.24 \pm 0.06^{e, f}$
Mean ± SD	3.89 ± 1.56	2.68 ± 1.46

Note: $^{a - h}$ =Means with different superscript letter along the column are significantly difference ($p<0.05$); ND= Not detected

3.6 Relationship between sucrose content and invertase activity

The correlation between the amount of sucrose in stingless bee honey and invertase activity was determined in Figure 1. From the regression analysis, there is some reliance between the amount of sucrose and invertase activity where the higher amount of sucrose resulting the lower invertase activity in honey. The amount of sucrose increased probably because of the factor of storage period and the honey was not matured enough due to sucrose was not wholly converted into fructose and glucose by enzyme invertase [18]. Five honey samples including *H. Itama* and *G.Thoracica* had low sucrose level and almost none invertase activity, thus it seems hard to conclude that these samples are lower in quality. The amount of the invertase in honey depends on many factors such as the condition of a bee colony, the age of the bees, food, temperature and intensity or type of honey flow [15].

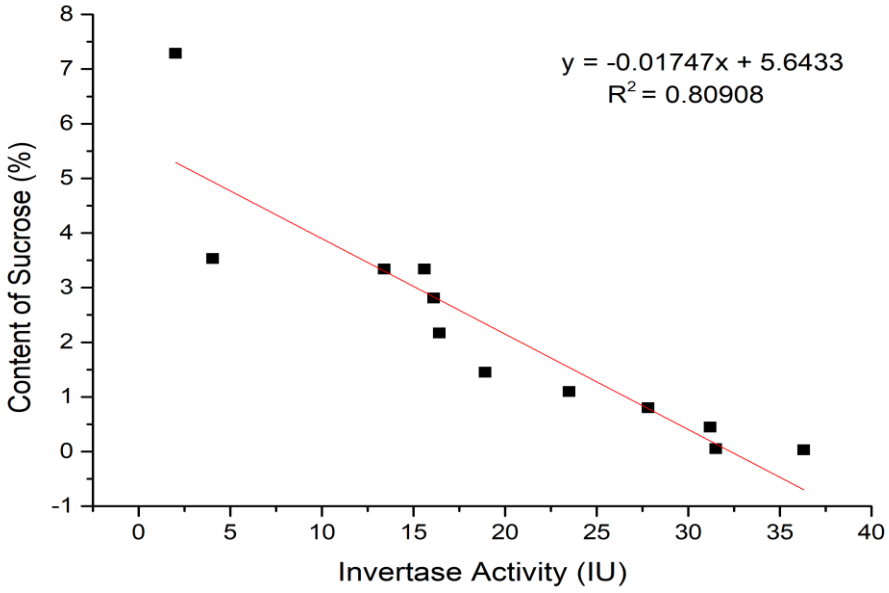

Figure 1. Influence of invertase activity on the content of sucrose in stingless bee honey samples from Malaysia

4. Conclusion

Seventeen samples of stingless bee honey collected from local market in Malaysia were analysed for their sugar profile and enzyme activity. From all commercial samples, six samples including C2, C3, C6, C11, C12 and C13 have similar characteristic when compare with harvested honey *H. Itama* and *G.Thoracica*. This result may suggest that these samples are good quality honey. The rest of commercial honey (9 samples) shows high value of glucose and sucrose content and lower enzyme activity which may indicate poor honey processing and possibility of adulteration. Since diastase and invertase result are lower than the international standard of *Apis mellifera* honey, we can conclude that enzyme activity in stingless bee honey are lower compare to *Apis mellifera* honey. This study has revealed that there is difference between good and adulterated honey however in future more samples can be added so that a decent data on sugar and enzyme content in stingless bee honey can be proposed and established which it may help the consumers from purchasing adulterated honey.

5. Acknowledgement

This study was financially supported by University Malaysia Pahang (Grant no: RDU 170332). We also would like to thank Aqif Kelulut Farm for assisting us in stingless bee honey collection.

6. References

[1] de Sousa J M B, de Souza E L, Marques G, Benassi M de T, Gullón B, Pintado M M and Magnani M 2016 Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi-arid region *LWT - Food Science and Technology* **65** 645–51

[2] Guo W, Liu Y, Zhu X and Wang S 2011 Dielectric properties of honey adulterated with sucrose syrup *Journal of Food Engineering* **107** 1–7

[3] Chanchao C 2000 Antibiotic and Organoleptic Properties of Honey from Apis dorsata(Giant Honeybee) and Trigona laeviceps (Stingless Bee)

[4] Biluca F C, Della Betta F, De Oliveira G P, Pereira L M, Gonzaga L V, Costa A C O and Fett R 2014 5-HMF and carbohydrates content in stingless bee honey by CE before and after thermal treatment *Food Chemistry* **159** 244–9

[5] Guerrini A, Bruni R, Maietti S, Poli F, Rossi D, Paganetto G, Muzzoli M, Scalvenzi L and Sacchetti G 2009 Ecuadorian stingless bee (Meliponinae) honey: A chemical and functional profile of an ancient health product *Food Chemistry* **114** 1413–20

[6] Pascual-Maté A, Osés S M, Fernández-Muiño M A and Sancho M T 2018 Methods of analysis of honey *Journal of Apicultural Research* **57** 38–74

[7] Rao P V, Krishnan K T, Salleh N and Gan S H 2016 Biological and therapeutic effects of honey produced by honey bees and stingless bees: A comparative review *Brazilian Journal of Pharmacognosy* **26** 657–64

[8] Anklam E 1998 A review of the analytical methods to determine the geographical and botanical origin of honey *Food Chemistry* **63** 549–62

[9] Kaškoniene V, Venskutonis P R and Čeksteryte V 2010 Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania *LWT - Food Science and Technology* **43** 801–7

[10] Gangwar S K 2016 Honey Physio-Chemical Parameters and Its Application With Reference To Ethiopia *International Journal of Science and Nature* **7** 16–24

[11] El Sohaimy S A, Masry S H D and Shehata M G 2015 Physicochemical characteristics of honey from different origins *Annals of Agricultural Sciences* **60** 279–87

[12] Mustafa M Z, Yaacob N S and Amrah S 2018 Reinventing the Honey Industry : Opportunities of the Stingless Bee *The Malaysian Journal of Medical Science:MJMS* **25** 1–5

[13] Malaysian Standard 2017 Kelulut (Stingless Bee)-Specification

[14] Bogdanov S 2009 Harmonised Methods of the International Honey Commision, *Bee Product Science*, 1–62

[15] Vorlov L and Piidal A 2002 Invertase and Diastase Activity in Honeys of Czech Provenience *Acta universitatis agriculturae et silvicuturae Mendelianae brunensis* **5** 57–66

[16] Buba F, Gigado A, Shugaba A 2013 Analysis of Biochemical Composition of Honey Samples from North-East Nigeria *Biochemistry & Analytical Biochemistry* **2** 139

[17] Suntiparapop K, Prapaipong P and Chantawannakul P 2012 Chemical and biological properties of honey from Thai stingless bee (*Tetragonula leaviceps*) *Journal of Apicultural Research* **51** 45–52

[18] Nascimento A, Marchini L, Carvalho C, Araújo D, Olinda R and Silveira T 2015 Physical-Chemical Parameters of Honey of Stingless Bee (Hymenoptera: Apidae) *American Chemical Science Journal* **7** 139–49

[19] Kek S P, Chin N L, Tan S W, Yusof Y A and Chua L S 2017 Classification of Honey from Its Bee Origin via Chemical Profiles and Mineral Content *Food Analytical Methods* **10** 19–30

[20] Boussaid A, Chouaibi M, Rezig L, Hellal R, Donsì F, Ferrari G and Hamdi S 2013 Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia *Arabian Journal of Chemistry* **11** 265-74

[21] Radia D, Azzedine C, Nicoletta D, Alice B, Chiara M, Albino G and Franco M 2015 Physicochemical parameters and antibiotics residuals in Algerian honey *African Journal of Biotechnology* **14** 1242–51

[22] White J W and Doner L W 1980 Honey Composition and Properties *Beekeeping in the United*

States Agriculture hand Book No335 1–12
[23] Codex Alimentarius Commission 2001 Standard For Honey *Codex Stan* **12** 1–8
[24] Chuttong B, Chanbang Y, Sringarm K and Burgett M 2016 Physicochemical profiles of stingless bee (Apidae: Meliponini) honey from South East Asia (Thailand) *Food Chemistry* **192** 149–55
[25] Bogdanov S, Lüllmann C, Martin P, von der Ohe W, Russmann H, Vorwohl G, Oddo L P, Sabatini A-G, Marcazzan G L, Piro R, Flamini C, Morlot M, Lhéritier J, Borneck R, Marioleas P, Tsigouri A, Kerkvliet J, Ortiz A, Ivanov T, D'Arcy B, Mossel B and Vit P 1999 Honey quality and international regulatory standards: review by the International Honey Commission *Bee World* **80** 61–69
[26] Pasias I N, Kiriakou I K and Proestos C 2017 HMF and diastase activity in honeys: A fully validated approach and a chemometric analysis for identification of honey freshness and adulteration *Food Chemistry* **229** 425–31
[27] Finola M S, Lasagno M C and Marioli J M 2007 Microbiological and chemical characterization of honeys from central Argentina *Food Chemistry* **100** 1649–53
[28] Alqarni A S, Owayss A A, Mahmoud A A and Hannan M A 2014 Mineral content and physical properties of local and imported honeys in Saudi Arabia *Journal of Saudi Chemical Society* **18** 618–25
[29] Dimins F, Kuka P and Cakste I 2008 Content of carbohydrates and specific rotation angle of honey *Foodbalt - 3rd Baltic Conference on Food Science and Technology* 121–5
[30] Assia A and Ali L 2015 Enzymes activities, hydroxymethylfurfural content and pollen spectrum of some Algerian honey *African Journal of Agricultural Research* **10** 613–22
[31] Buba F, Gigado A, Shugaba A 2013 Physicochemical and Microbiological Properties of Honey from North East Nigeria *Biochemistry & Analytical Biochemistry* **2** 61-67