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

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**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



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production of impure honey. In a year, stingless bee only can produce up to 4 kg honey per colony compared to *Apis mellifera* 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 ( $\text{KH}_2\text{PO}_4$ ), disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ ), sodium chloride (NaCl), and hydrochloric acid (HCl) were purchased from Sigma-Aldrich (USA), and p-Nitrophenyl- $\alpha$ -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  $5\mu\text{ NH}_2$  80A, 250 x 4.6 mm, Phenomenex Inc, USA) and detected by refractive Index detector (RID) operated at 35°C. 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 40°C. 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 40°C.

### 2.5.2 Invertase Activity

The invertase activity was determined by following the method of International Honey Commission [14]. Substrate solution; p-Nitrophenyl- $\alpha$ -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  $56.93 \pm 16.5$  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  $7.29 \pm 0.18$  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  $\pm$  standard deviation, n = 3).

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

Note: <sup>a - k</sup> = 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  $3.89 \pm 1.56$  (Table 2). According to Codex Alimentarius [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<sup>-1</sup>) or as invertase number (IN), where 1 IN is equal to 7.344732 IU kg<sup>-1</sup> [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  $2.77 \pm 1.22$  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. Thora* 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].

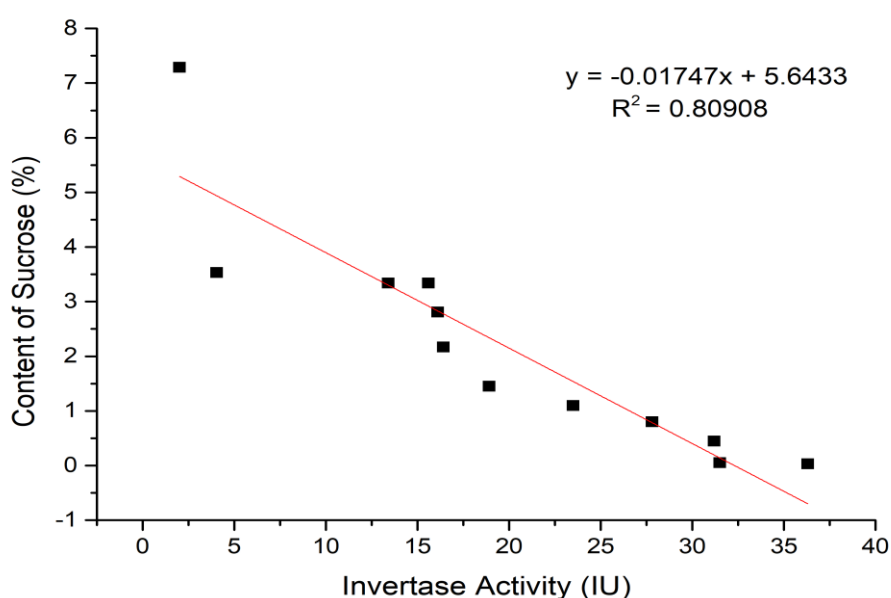
**Table 2.** Summary of predominant enzyme activity of various stingless bee honey samples from Malaysia (mean  $\pm$  standard deviation, n = 3).

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

Note: <sup>a-h</sup> = 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

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