

## Physicochemical properties, sensory acceptance and glycaemic index of processed stingless bee honey and processed honeybee honey

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### Abstract

One of the major factors in chronic disease is excessive sugar consumption and high glycaemic index (GI) food intake. The present study was carried out to compare the GI and glycaemic load (GL) of processed stingless bee honey (P-SBH) and processed honeybee honey (P-HBH) in addition to their physicochemical properties. The physicochemical were determined following the International Honey Commission standard procedures. For the GI and GL determination, 10 subjects were given honey containing 25 g of available carbohydrate in 250 mL of water. The blood glucose responses were based on incremental area under the curve, IAUC, and compared to that of 25 g available carbohydrate from pure glucose. The findings revealed that P-HBH had higher total sugars (g/100 g) content (89.18) than P-SBH (62.38). In terms of sensory acceptance, P-SBH is more preferred as the overall scores (4.6±0.8 vs 2.5±0.7), sweetness (4.2±1.0 vs 2.2±0.6), colour (5.6±0.5 vs 1.8±0.4) and odour (5.1±0.3 vs 2.0± 0.0) were significantly higher than P-HBH ( $p<0.05$ ). However, the aftertaste of P-HBH was more apparent (1.1±0.4) than that of P-SBH (4.2±0.4). The results showed a significant difference ( $p<0.05$ ) in GI between P-SBH (91±42) and P-HBH (67±23). While in terms of GL, no significant difference was documented ( $p>0.05$ ) and both processed honey can be classified as low GL foods. In conclusion, this study showed that higher total sugar content in P-HBH does not affect the GI and can be consumed in moderate amounts following the suggested carbohydrate allowance.

## 1. Introduction

Honey is a natural sweetener and a traditional medicinal agent. The international body of glycaemic index (GI) indicates that honey has a GI value ranging from 32-87 (Rajab *et al.*, 2017). Importantly, several studies show the blood glucose response of honey is lower than comparable amounts of pure sugar (Rajab *et al.*, 2017). There are two types of honey, which are honeybee honey and stingless bee honey. Stingless bee honey, which is produced by bees of the genus *Meliponines*, differs from honeybee honey, which is produced by the bees of the genus *Apis* (*i.e.*, the honey bee) in terms of colour, taste, moisture, viscosity and other features (Rao *et al.*, 2016).

The physicochemical properties such as moisture, ash, pH and free acidity, electrical conductivity (EC), and sugar content always vary between stingless bee honey and honeybee honey. Moisture content in honey represents the stability of honey against fermentation and granulation. Low moisture content protects honey from damaging microbiological activity (Azonwade *et al.*, 2018). Moreover, monosaccharides represent about 75% of the sugars found in honey, along with 10–15% disaccharides and small amounts of other sugars. Honey contains a small amount of acid that has been added by the bees. The acid is important for the taste of honey and also to discriminate the honey according to its botanical or geographical origin, and the main acid in the honey is gluconic acid, a product of glucose oxidation by glucose

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oxidase (Da Silva *et al.*, 2016). Sensory analysis also plays a part in the measurement of physicochemical properties, as it determines the overall acceptability among honey consumers. The sensory analysis combined with other composition elements is also a complementary tool to evaluate the botanical origin of honey (Rodríguez *et al.*, 2019).

Honey should not be heated or processed to such an extent that would affect its essential composition and reduce its quality (Guo *et al.*, 2011). There are several methods used to process the honey, namely, straining (Subramanian *et al.*, 2007), filtration (Belay *et al.*, 2017), conventional thermal treatment (Fauzi, 2014), microwave (Moliné *et al.*, 2015) and infrared heat processing (Subramanian *et al.*, 2007). The demand for better quality honey is on the rise as honey is being consumed now for its health benefits. Therefore, efforts are being made to look for alternatives to the conventional thermal process to produce higher-quality honey (Subramanian *et al.*, 2007). Processing is also necessary to cope with the chaos that exists in international legislation by adopting minimum standards as mandatory for all countries (Thrasylvoulou *et al.*, 2018). However, the processing causes changes in its chemical constituents while previous studies for example by El-Sohaimy *et al.* (2015) only focus on raw or unprocessed honey, but not the processed honey whereby most of the honey available in the market is processed.

The GI categorizes foods quantitatively based on the blood glucose-raising potential of the carbohydrates in the foods or the rate of carbohydrate absorption in the blood (Rosli, 2015). Different carbohydrate-rich foods have different effects on blood glucose levels, while the amount of carbohydrates consumed has the greatest influence on blood glucose levels after meals (Rajab *et al.*, 2017). GI levels can be classified as high-GI (> 70), moderate-GI (>55 - < 70) or low-GI (< 55) (Eleazu, 2016). Generally, a low-GI diet reduces mean blood glucose concentrations and insulin secretions in clinical trials in normal and diabetic participants. Therefore, compared with high-GI diets, low-GI diets offer a good means of reducing the rapidly-increasing rates of diabetes in many countries (Atayoğlu *et al.*, 2016).

The glycaemic response to food is dependent not only on GI but also on the total amount of carbohydrates consumed, leading to the concept of glycaemic load (GL). GL accounts for the number of carbohydrates in food. The amount of each gram of carbohydrate in food raises blood glucose levels and is classified into low (< 10), intermediate (11-19), and high (>20) (Eleazu, 2016). Many studies have demonstrated the health benefits of low GI or GL diets, including naturally occurring and

minimally processed carbohydrates such as cereals, vegetables, and fruit. These foods have numerous qualities besides their immediate impact on postprandial glycaemia as a basis to recommend their consumption (Venn and Green, 2007).

Thus, this study aimed to investigate the glycaemic GL, sensory acceptance and physicochemical properties of processed stingless bee honey (P-SBH) and processed honeybee honey (P-HBH).

## 2. Materials and methods

### 2.1 Sample preparation

Samples of two kinds of honey, P-SBH and P-HBH were selected for this study. The honey was obtained from a honey farm in Marang, Terengganu. Prior to the process of dehydration, the moisture content was tested using a digital refractometer (NR101, Spain). Then, the honey was sieved and left in a storage container for 24 hrs. After that, the moisture was checked every 4 hrs until it was reduced to 20.00% by using a dehydrator (Dewoods L8125PXL TU- CE). Lower moisture limits of no more than 20 g/100 g elongate honey shelf life, which has been proposed by some countries for the revision of the Codex Alimentarius 2001 (El-Sohaimy *et al.*, 2015).

### 2.2 Physicochemical analysis

Sugar content was analysed using the method from the International Honey Commission (IHC, 2009) and Raja Nurfatin *et al.* (2021). Each mixture was analysed using High-Performance Liquid Chromatography (HPLC). The following conditions were found to give good separation for a column of Xbridge<sup>®</sup> Amide (3.5 µm, 4.6 × 250 mm; Waters, Ireland; Catalogue No: 186004870): flow rate of 1.3 mL/min; mobile phase of acetonitrile, water (80:20, v/v); column and detector temperature of 30°C; and a sample volume of 10 µL. The sugars in the honey were identified and quantified by comparison of the retention times and the peak area of the honey sugars with those of standards sugar (D-(+)-glucose, D-(-)-fructose, sucrose, D-(+)-maltose monohydrate and D-lactose monohydrate, all from Sigma). The mass percentage of the weight of sugar, the weight of fructose, glucose, maltose, and so on were determined in terms of g/100 g.

The moisture, ash, pH, free acidity, and EC were determined following Raja Nurfatin *et al.* (2021) based on the method from the International Honey Commission IHC (2009). For the sample preparation, honey was homogenized and put in a flask. The flask was closed, and the sample was placed in a water bath at 50°C (±0.2) until all the sugar crystals were dissolved.

### 2.3 Glycaemic index and load determination

The GI value for each honey was determined by following the protocol from the International Organization of Standardization ISO 26642:2010 (International Organization for Standardization, 2010). Eligible subjects that fulfilled the inclusion criteria, including the oral glucose tolerance test (OGTT), were selected to participate in this study. A total of 10 subjects (all female) participated in this study following Chepulis and Francis (2012). The age of subjects ranged from 18-25 years old (Radhwan *et al.*, 2018) with the body mass index (BMI) ranging between 19.5- 26.5 kg/m<sup>2</sup>. All subjects were also free from diabetes and did not suffer from any chronic diseases such as cardiovascular disease, cancer, cirrhosis and mental disorders. In addition, they had not participated in any clinical trial in the past three months. These screening methods are in line with standard GI testing criteria.

Prior to the GI test, all subjects were subjected to an OGTT to ensure that they met the inclusion and exclusion criteria. The subjects were asked to fast overnight for 10 to 12 hrs. The subjects came to the test venue at a specific time. After that, they were given 50 g of pure glucose (Glucolin) in 250 mL of water and asked to completely consume the glucose solution within 5 mins, and the blood glucose was taken at 120 mins. The subject who fulfilled the inclusion criteria of normal OGTT reading was selected. For the GI study, subjects were initially given 25 g of glucose that was dissolved in 250 mL of water. Blood glucose samples were taken at each time point (0, 30, 60, 120 mins) from the initial time of consumption. For the honey test samples, a similar procedure was followed. Again, after 10-12 hrs of overnight fasting, the subjects' blood glucose levels were taken. Then, they were given a calculated amount of honey containing 25 g of available carbohydrates in 250 mL of water. Thus, in order to meet the 25 g of available carbohydrate content, 28.03 g of P-HBH and 40.08 g of P-SBH were applied. GI and GL were calculated following the recommendation from the Food and Agriculture Organization/World Health Organization (1998).

$$GI = \frac{AUC \text{ for test food}}{AUC \text{ for reference food}} \times 100$$

$$GL = \frac{GI \text{ of carbohydrate} \times \text{grams of carbohydrate per serving food}}{100}$$

The present study was approved by the Universiti Malaysia Terengganu Ethics Committee (UMT/JKEPHMK/2020/46). Written consent forms were obtained from all subjects.

### 2.4 Sensory acceptance

The sensory acceptance of honey was performed following the procedures of Sharif *et al.* (2017). Sensory acceptance analysis was conducted after the respondents completed the GI study, which included 10 volunteer panellists recruited among them. A seven-point hedonic scale was used in this evaluation test. The scores were ranked from 1, dislike extremely, to 7, like extremely. The attributes that the panellist evaluated included colour, odour, aftertaste, texture, and overall acceptance.

### 2.5 Statistical analysis

The duplicate data obtained in this study were expressed as mean(standard deviation). Parametric tests included an independent t-test to compare the physicochemical properties and sensory acceptance between P-SBH and P-HBH, and a one-way ANOVA with post-hoc Tukey's to determine any significant difference for GI and GL involving glucose, processed SBH and processed HBH. Statistical Package for the Social Science (SPSS for Windows, Version 16.0 Chicago, SPSS Inc.) software was used, and in all analysis,  $p < 0.05$  was taken to indicate significance.

## 3. Results and discussion

### 3.1 Physicochemical properties of processed honey

In general, the physicochemical properties of P-HBH were comparable with the published data in the International Honey Standards specified by Codex Alimentarius Standard, 2019. Besides that, the physicochemical properties of processed P-SBH were comparable to Malaysian Quality Standards, 2017.

As shown in Table 1, the results show that the moisture contents of both samples were similar because the honey was each processed to reduce its moisture to 20.00%. A study showed that water content depends on the botanical origin of the honey, the level of maturity of the hive, processing techniques and storage conditions (Da Silva *et al.*, 2016). Honey with high water content is more likely to ferment (Bogdanov *et al.*, 2015). Thus, the Codex Alimentarius Standard and Malaysian Standards (2017) have set limits for moisture content of not more than 20.00% to prevent fermentation. In the present study, raw honeybee honey and raw stingless bee honey were processed with a dehydrator until the moisture reached 20.00% to meet this standard of quality.

As stated in the Codex, the acceptable range for the ash content of honey ranges between (0.6–1.2 g/100 g). However, the P-HBH was less than the range that was required by that international standard, which is 0.39 g/100 g. This result may indicate that the mineral content

Table 1. Physicochemical properties of P-SBH and P-HBH

Components (g/100 g)	Processed Stingless Bee Honey (P-SBH)	Processed Honeybee Honey (P-HBH)
Moisture	20.00 (0.00)	20.00 (0.00)
Ash	0.44 (0.05) <sup>a</sup>	0.39 (0.01) <sup>a</sup>
pH	3.07 (0.02) <sup>b</sup>	3.27 (0.02) <sup>a</sup>
Free Acidity (meq/kg)	83.17 (1.76) <sup>b</sup>	111.67 (1.53) <sup>a</sup>
Electrical conductivity (mS/cm)	0.86 (0.05) <sup>a</sup>	0.82 (0.03) <sup>a</sup>
Total reducing sugars content	62.38	89.18

Values are reported in mean (SD). Values with different letter superscripts are significantly different ( $p < 0.05$ ) by independent t-test. For total reducing sugars, data are presented from a single injection of HPLC analysis.

of the P-HBH was lower (Azonwade *et al.*, 2018). For P-SBH, the ash content of 0.44 g/100 g was aligned with the suggested quality standards of Malaysia (max 1.0 g/100 g). Usually, the ash content of honey correlates to its mineral content, such as potassium, sodium, magnesium, and calcium, and is influenced by the composition of the source plant nectar (Nordin *et al.*, 2018).

The average pH of P-HBH in the present study was 3.27, which is lower than the standard limit (pH 3.40–6.10) stated by Codex Alimentarius, 2019. Low pH values indicate more acidity, as the lower the pH of the honey, the better its ability to inhibit the presence and growth of microorganisms (Fatima *et al.*, 2018). For P-SBH, the pH result is 3.07, which matches the Malaysian Standard of an acceptable pH range of stingless bee honey of 2.5 to 3.8.

The Codex Alimentarius Standard for honeybee honey permits a maximum value of 50.00 meq/kg for free acidity, but the results for P-HBH (111.67 meq/kg) did not comply with this international standard. The free acidity of P-SBH was 83.17 meq/kg, and there is no standard applicable in the Malaysian Standard for P-SBH. Higher values of free acidity may indicate the fermentation of sugars into organic acids in honey. In addition, higher free acidity might also indicate that the honey samples have high mineral content (El Sohaimy *et al.*, 2015).

The Codex Alimentarius standard sets a limit for the EC of honeybee honey of not more than 0.8 mS/cm. However, the EC value for P-HBH (0.82 mS/cm) was very close to the standard limit. The EC for P-SBH was 0.86 mS/cm; however, this standard is not mentioned in the Malaysian Standard. The measurement of EC depends on the ash and acid content of honey, and higher ash and acid content may contribute to a higher result of EC (El Sohaimy *et al.*, 2015). Furthermore, the concentration of minerals, salts, organic acids and proteins in honey is also directly related to its EC (Nordin *et al.*, 2018).

Finally, according to the standards of the Codex Alimentarius Committee (2019) on sugars, the minimum

amount of reducing sugars is 60.00%, and this standard complies with the result of P-HBH (89.18%). In the Malaysian Standard, stingless bee honey should not have a sum of fructose and glucose levels of more than 85 g/100 g, and this limitation was matched by the P-SBH (62.38%). The results of sugars content analysis indicate that sugars are the major constituents in both kinds of honey, in agreement with Azonwade *et al.* (2018). In general, the sugar composition of honey is affected by the types of flowers used by the bees, as well as regions and climate conditions. The quality criterion also depends on total sugar content (Da Silva *et al.*, 2016).

### 3.2 Sensory acceptance of honey

Sensory evaluation is a scientific discipline that analyses the responses of humans to the composition of food and drinks, such as appearance, touch, odour, texture, temperature and taste. Sensory evaluation of honey enables us to distinguish the botanical origin of the honey and to identify and quantify certain defects (Saludin *et al.*, 2019). Figure 1 shows the sensory evaluation of both kinds of honey.

Colour is the first attractive attribute of honey, and it is very important for commercialization. It is an important parameter in the quality, acceptance, and preference of consumers to buy and consume honey (Da Silva *et al.*, 2016). It is most likely that acceptability

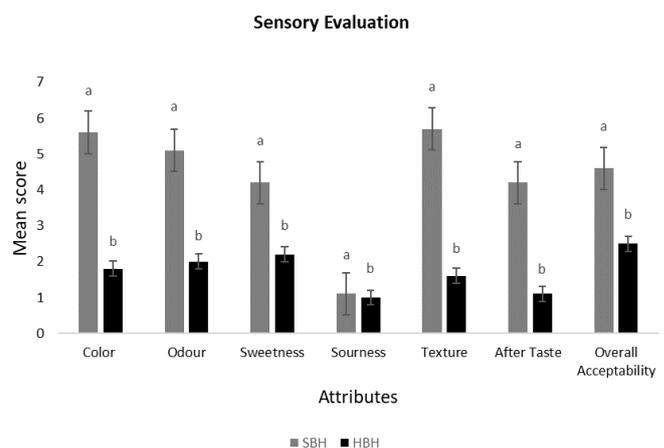


Figure 1. Sensory evaluation of processed stingless bee honey and P-HBH. Bars are presented as mean with different superscript notations are significantly different ( $p < 0.05$ ). Data were analysed with Independent T-test.

scores were affected by panellist bias based on the physical properties of P-HBH that had been crystallized (Srinual and Intipunya, 2009).

As described by Saludin *et al.* (2019), the sour taste of honey was one of the factors that influenced consumer preferences and purchases. According to Nascimento *et al.* (2015), stingless bee honey has a lower sugar content, which influences its less-sweet and more-sour taste. Half of the panellists accepted the sour taste and half did not, leading to the lower mean score for this attribute. Moreover, for P-HBH, Zamora and Chirife (2006) found that changes in water activity of honey during crystallization allow the growth of osmophilic yeasts such as *Saccharomyces* spp. leading to an increased level of yeast growth and fermentation, which contribute to the sour taste of honey.

For viscosity, there was a significant difference between P-SBH and P-HBH ( $p < 0.05$ ). In a recent study by Saludin *et al.* (2019), the viscosity of the stingless bee honey was the major factor influencing the consumers' purchasing decisions regarding stingless bee honey quality. The panellists did not prefer the firm texture caused by crystallization. As crystallization proceeded further, the processed honey was less acceptable to the taste of panellists. In addition, the crystallization of honey affects its sensory acceptability and physicochemical properties (Srinual and Intipunya, 2009).

### 3.3 Glycaemic index and glycaemic load

Figure 2 shows the values of GI for glucose and the two processed kinds of honey samples. P-SBH had a higher GI value (91) than P-HBH (67) even though the amount of total carbohydrates given to the respondents are the same (25 g).

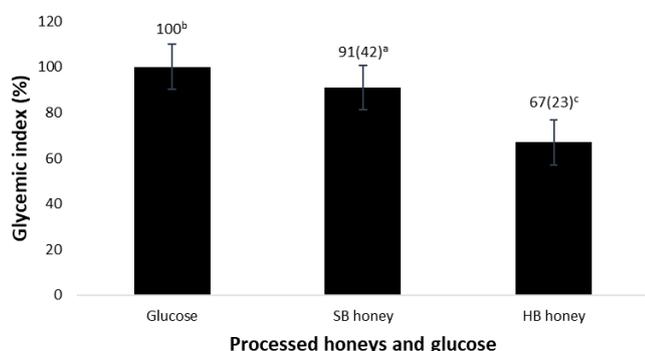


Figure 2. GI for P-SBH, P-HBH and glucose. Values are mean $\pm$ SD. Values with different superscripts are significantly different ( $p < 0.05$ ).

GI is defined as the incremental area under the curve of blood glucose response (IAUC) following ingestion of 50 g of available carbohydrates in the test product as a percentage of the corresponding area, following an

equivalent amount of carbohydrate from a reference product such as glucose (Arvidsson-lenner *et al.*, 2016). There is an option to use 25 g, instead of 50 g of available carbohydrate, which raises an ethical question. According to a meta-analysis, the relative risk of developing type 2 diabetes is 0.97 for every 50 g of carbohydrates consumed (Greenwood *et al.*, 2013). In the present study, 25 g of carbohydrate was used instead of 50 g of carbohydrate because it has been reported that the dose-response curve of IAUC glucose response is linear in the range of 25 g to 50 g of carbohydrates.

The results of this study showed that the total amount of honey exhibited significant effects on blood glucose response, even though the total amount of reducing sugars consumed was the same. The sugar content in honey might not be the main reason causing the GI differences because their profiles were very similar to each other. The present study is supported by a previous study that found no significant correlations between GI and either total sugars, fructose level, organic acid content, pH, or osmolality (Chepulis and Francis, 2013). In addition, Ischayek and Kern (2006) also documented no relationship between the GI and the fructose-to-glucose ratio, indicating that small differences in fructose-to-glucose ratios do not substantially have an impact on the honey GI. Some other studies have also reported that although honey is composed mainly of varying simple sugars, it does not differ significantly from glucose as a reference food in its glycaemic response (Rajab *et al.*, 2017). Table 2 shows the sugar content per amount of honey (25 g available carbohydrate) given to the respondents.

Even though honey usually contains a high amount of fructose, it also contains high amounts of glucose. Glucose itself increases fructose absorption in the small intestine, and this effect becomes more prominent where an equal amount of fructose and glucose are consumed (Kyaw *et al.*, 2011). Hence, the glucose content of honey is an important factor in determining its usefulness of honey for diabetes patients (Erejuwa, 2014). Honey requires lower levels of insulin as compared to regular white sugar and does not raise blood sugar levels as substantially as table sugar, and additionally has a lower GI than sugar (Fessenden, 2014).

Furthermore, the reason behind the differences in GI for P-SBH (91) and P-HBH (67) might be the amount of honey ingested. Two studies stated that the hypoglycaemic effect of honey is dose-dependent (Erejuwa, 2014). As shown in Table 2, the dose given to the respondent for P-SBH (40.08 g or 4 spoons) was higher than P-HBH (28.03 g, or 3 spoons). This statement is in agreement with a study by Deibert *et al.*

(2010), which found that diet therapy for diabetes does not recommend the consumption of a large amount of simple sugar separately in one meal without taking them with any other foods, as it will affect greater in blood glucose response or GI. The difference in GI may be caused by the dose of each honey given to the respondents and corresponding to the serving size or whether odd (3 spoons) or even (4 spoons) numbers, might affect the outcomes as reported by Raja Nurfatin *et al.* (2021). Some of these studies used a single dose of honey thus, the results cannot accurately demonstrate honey's suitability for long-term use (Sadeghi *et al.*, 2019). Therefore, long-term research is needed to evaluate in detail the metabolic effects of dose consumption of honey with different GIs on healthy individuals.

Table 2. Sugars content per amount of honey (containing 25 g available carbohydrate) that was given to the respondents

Sugars content	40.08 g of P-SBH	28.03 g of P-HBH
Fructose	11.96 g	12.49 g
Glucose	11.78 g	11.47 g
Sucrose	0.81 g	0.43 g
Maltose	0.37 g	0.48 g
Lactose	0.03 g	0.10 g
Total sugar	24.95 g (~25.0)	24.97 g (~25.0)

The GI of P-HBH was 67, categorized under the acceptable range of the other honey in the other countries as the five Jordanian floral honey types ranged from 45.4 (Christ thorn) to 85.5 (Spanish thistle). The high GI of Spanish thistle honey may be explained by the high variability within their volunteers and the honey being tested by only four volunteers (Rajab *et al.*, 2017). The GI values of minimally processed monofloral Turkish honey fall within the range of 44.9 - 69.1, and this GI range also matches the GI value of P-HBH (67) (Atayoğlu *et al.*, 2016). In the present study, the P-HBH was comparable to the GI value of processed honey of *Apis mellifera* (63), *Apis cerana* (67), and *Apis dorsata* (62). However, the P-SBH is higher at 91 than the P-SBH of *Trigona iridipennis*, with GI 56 (Krishnasree, 2017). Only a few studies have considered the GI of P-SBH previously.

Figure 3 shows that the GL of two processed honey was not significantly different between P-SBH and P-HBH ( $p > 0.05$ ). The significant difference between the two processed honey and glucose could be due to glucose's medium GI while P-SBH and P-HBH were categorized as low GL foods since their GL values are less than 10.

For an individual food, it is intuitively obvious that the GL will be more relevant than the GI (Willett *et al.*, 2002). Thus, P-SBH and P-HBH can be considered as

food that does not produce substantial postprandial blood glucose and insulin responses as the GI is low.

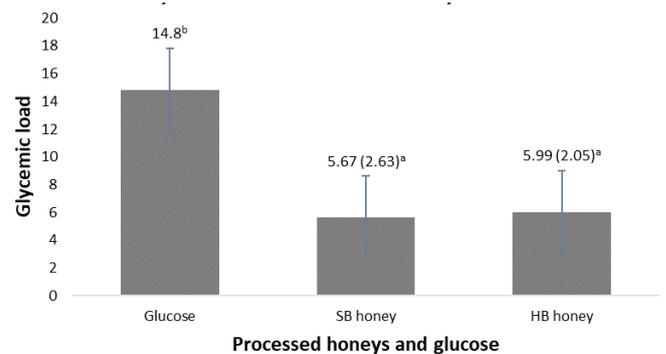


Figure 3. GL for P-SBH, P-HBH and glucose. Values are mean(SD) with different superscripts are significantly different ( $p < 0.05$ ).

#### 4. Conclusion

The GI for P-SBH was categorized as having a higher GI than that of P-HBH, which had a moderate GI. However, in terms of GL, both P-SBH and P-HBH are considered low-GL foods, even though P-HBH has higher total sugar content. Therefore, the GI and GL values are not necessarily dependent on total sugar content. Sensory acceptance shows that most panellists preferred P-SBH over P-HBH.

#### Conflict of interest

The authors declare no conflict of interest.

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