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MICROBIOLOGICAL QUALITY AND pH CHANGES OF HONEY PRODUCED BY STINGLESS BEES, *Heterotrigona itama* AND *Geniotrigona thoracica* STORED AT AMBIENT TEMPERATURE

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ABSTRACT

The Meliponini honey is one type of stingless bee honey. Although honey has been widely used as a natural remedy, understanding on how storage affect the microbiological quality and pH in stingless honey is still scarce. This study was carried out to determine the microbial quality of honey produced by stingless bees, *Heterotrigona itama* and *Geniotrigona thoracica* during ambient storage for 6 weeks. The changes in microbiological analysis was determined for Aerobic Plate Count (APC), yeast count and *Bacillus* count. Lactic acid bacteria (LAB) were isolated using three types of media, namely, MRS agar, MRS agar with 0.8% $CaCO_3$ and MRS agar with 1% glucose. Detection of *Clostridium* was done using anaerobic egg-yolk agar and cook meat medium. Results showed that Aerobic Plate Count ranged between 10^2 to 10^5 CFU/g for both samples. However, yeast count showed the range between 10^2 to 10^6 CFU/g, which higher than APC. Both honey samples showed low in *Bacillus* count. The LAB counts on three media were significantly higher for the first month of storage, then decreased gradually after fifth week. Storage of honey had reduced the pH in both honey samples to pH 2. *Clostridium* was not detected in all samples.

Key words: Microbiological quality, pH change, ambient storage, Lactic Acid Bacteria, stingless bee honey

INTRODUCTION

Meliponini is a stingless bee that produces honey that include *Heterotrigona itama, Geniotrigona thoracica* and *Trigona fulviventris.* A stingless bee is a type of eusocial insects that live in multi-generational family groups in which the majority of individuals cooperate for reproductive group members. Their colonies range from a few dozens to 100,000 or more adult workers that live in the tropical and subtropical regions of the world (Michiner, 2000). They produce less honey compared to *Apis mellifera.* However, stingless bees have good quality, and reported to have antitumoral, antimicrobial and antioxidant activities (Gómez-Caravaca *et al.,* 2006). The physico-chemical and microbiological properties of honey samples obtained from Ibadan, Nigeria have been reported (Adenekan *et al*., 2010). However, there is no study reports on the effect of physico-chemical and microbiological properties of Meliponini honey during storage. Demands on for Meliponini honey in Malaysia is increasing due to its reported health benefits and treatment for various diseases.

Spore forming aerobes and anaerobes are reported as predominant microflora of honey, but other genera of microorganisms such as Lactic Acid Bacteria (LAB) (Aween *et al.,* 2012a; Aween *et al.,* 2012b; Bulgasem *et al.,* 2015, Bulgasem *et al.,* 2016), yeast and fungi were also detected in honey (Barnett *et al.,* 1990) either as part of existing microflora or contaminated from environment

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(Olaitan *et al.,* 2007). The primary sources of microorganisms in honey could be from pollen, soil, dust, water, digestive tract of Meliponini, air and nectar which are difficult to control. Post-harvest microbial contamination in honey can also occur as a result of lack of concern on the hygiene of processing, handling and storage of honey. Air and dust are important sources of *Bacillus* sp., *Clostridium* sp. and *Micrococcus* species (Kacaniova *et al.,* 2007).

Most microbes found in honey are not dangerous to the consumer's health. Honey has inherent antimicrobial properties that can delay or inhibit growth of many microbes (Adenekan *et al*., 2010). It has been long recognised that quality of honey influenced by the seasonal variations, post-harvest handling of honeys and their storage conditions. However, there are some cases related to the infant botulism due to the presence of *Clostridium botulinum* in honey. For this reason, the FDA, the Centers for Disease Control and Prevention (CDC), and the American Academy of Pediatrics recommend not feeding honey to infants under one year old.

The stability and shelf life of honey is influenced by pH of honey during the extraction from the hives and storage (Terrab *et al.,* 2004). Changes in pH of honey indicates production of organic acids; this makes honey more sourish in taste and could affect consumers' perception of honey produced by stingless bee, *Heterotrigona itama* and *Geniotrigona thoracica* during storage for six weeks at ambient temperature (25 ± 2°C). Changes in pH and total counts of yeasts and lactic acid bacteria as well as detection of *Clostridium* sp. and *Bacillus* sp. were carried out to ascertain the microbiological quality.

MATERIALS AND METHODS

Honey samples

Meliponini honey samples were collected from Pusat Tunas Stevia Jabi, Besut, Terengganu located approximately 90 km from Kuala Terengganu. The hives were man-made wooden hive. Honey samples were collected by using a suction pump and placed in the previously sterilised blue cap bottle. The honey samples were kept and protected from sunlight to prevent any degradation of the honey during transportation to the laboratory as suggested by Guerrini (2009).

Storage of honey samples

Each honey samples (50 mL/bottle) was distributed to seven previously sterilsed universal bottles and stored at ambient temperature (25 ± 2°C) in the dark. Changes in microbiological counts and pH was monitored at weekly intervals.

Determination of Aerobic plate count (PCA) in honey samples

Aerobic plate count was carried out following the methods described by Food and Drug Administration's Bacteriological Analytical Manual described by Merker (1998). Honey samples (10 g) was thoroughly mixed with 90 ml of 0.1% sterile buffered peptone water (Merck, Germany) in a stomacher bag using a stomacher (BagMixer, Interscience, France). Appropriate dilution was spread plated on the Plate Count Agar (Oxoid, UK) and incubated at 35°C for 24 h. A 24-h culture of pure selected isolates were Gram stained and the morphology was observed using a light microscope (Leica DM1000, Germany).

Determination of yeast count in honey samples

Honey samples (10 g) was mixed with 90 ml of 0.1% sterile buffered peptone water (Merck, Germany) before homogenizing in blue caps bottle. Appropriate dilution was spread plated on Yeast Malt extract Agar (Oxoid, UK) and incubated at 30°C for 24- 48 h. A 48 h culture of yeast isolates were stained with lactophenol blue and the morphology was observed by using a light microscope (Leica DM1000, Germany).

Determination of *Bacillus* count in honey samples

Honey samples (10 g) was added with 90 ml of 0.1% sterile buffered peptone water (Merck, Germany). Appropriate dilution of the samples was spread plated on the Mannitol Egg Yolk Polymyxin Agar (Oxoid, UK) and incubated at 35°C for 24–48 h.

Determination of lactic acid bacteria count from honey samples

LAB were isolated from honey samples following the method described by Aween *et al*. 2012a that involved pre-enrichment step before isolation on de Man Rogosa and Sharpe (MRS) Agar (Oxoid, UK). Honey sample (10 g) was aseptically transferred to 90 ml of MRS broth (Oxoid, UK) and incubated in a CO_2 incubator (Model ICO150 Memmeth, Germany) at 30°C for 24 h. A 100 µl of appropriate dilution in 0.1% saline was spread plated on three media, namely, de Man Rogosa Agar (MRS), MRS agar with 0.8% $CaCO_3$, and MRS agar with 1% glucose incubated at 30°C for 48 h in an anaerobic incubator (Model ICO150 Memmeth, Germany). Colonies with clear zone on MRS Agar with 0.08% $CaCO_3$ were considered presumptive LAB. A 24-h pure cultures were Gram stained and further tested for catalase activity using 4% hydrogen peroxide; cells producing gas bubbles were considered catalase positive. LAB are catalase negative organism.

Isolation of *Clostridium* sp. from both honey samples

Detection on the presence of *Clostridium* sp. was carried out as described by Küplülü *et al.* (2006). Aseptically, 25 g honey was diluted in 100 ml sterilized distilled water containing 1% Tween 80 (Merck, Germany) thoroughly mixed using a vortex. The mixture was heated at 65°C in water bath for 30 minutes to inactivate vegetative cells, then rapidly cooled in iced water. The mixture was centrifuged at 9000 x g, 4°C for 30 minutes. The precipitates were transferred into 9 ml cook meat media (Oxoid, UK), layered with sterile paraffin oil and incubated at 35°C for 7-10 days. After 7 days of incubation, each culture was examined for their turbidity and gas production. Cultures that have no turbidity and gas production considered as negative and were further incubation for additional 3 days to allow late germination of spores. After 10 days of incubation, cultures with no significant signs of growth were considered to be negative. Tubes that showed positive reactions were streaked onto Anaerobic Egg Yolk agar (Oxoid, UK). The plates were incubated at 35°C for 48 h in CO incubator (Model ICO150 Memmeth, Germany). At the end of incubation time, isolated typical colonies were re-streaked on two AEY agar plates; one incubated aerobically and the other one incubated anaerobically at 35°C for 48 h. Colonies that grew anaerobically were considered to be pure cultures of *Clostridium* spp. (Küplülü *et al.,* 2006). The pure *Clostridium* strains was obtained from original stock cultures supplied by Biolution Resources Sdn. Bhd, Kuala Lumpur. Typical colony showing an opaque precipitate around the colonies due to the degradation of lecithin in the egg yolk. The lipase enzyme hydrolyzes the fats within the egg yolk, which results in an iridescent sheen on the colony surface. These were the indicative of *Clostridium* sp. (Kuplulu *et al.,* 2006).

Determination of pH in both honey samples

The pH of sample was determined by using pH meter (Model 3505, Jenway, UK) at weekly intervals during 42 days storage period at ambient temperature (25 ± 2°C).

Statistical analysis

In order to evaluate significant differences for microbial counts and pH, paired t-test were used, and a significant level of α=0.05 was chosen. All the statistical analysis was performed using Minitab Version 14.0.

RESULTS

Aerobic plate count (APC) in two types of Meliponini honeys

Aerobic Plate Count (APC) can indicate the total population of microorganism in each storage days of honey. There were not significant differences ($p>0.05$) in Aerobic Plate Counts between *H. itama* and *G. thoracica* honeys at 6 weeks of storage. However, the APC in sample A (*H. itama*) had slightly higher count (3.76 $\log_{10}$ CFU/g) compared to sample B (*G. thoracica*). Table 1 shows the APC ($\log_{10}$ CFU/g) of both stingless bee honey from *H. itama* and *G. thoracica* at 0, 7, 14, 21, 28, 35, and 42 days. From this study, the lowest Aerobic Plate Count (APC) count was observed in both sample were 10^2 CFU/g during 21 and 42 days of storage whereas the highest APC 5-$\log_{10}$ CFU/g were observed at the beginning of the storage which at 7 days of storage. The APC count started to decrease after 7 days of storage until day 21 from 5-$\log_{10}$ CFU/g to 2-$\log_{10}$ CFU/g. Then, after 21 days of storage, the APC count increased again to 35 days of storage from 2-$\log_{10}$ CFU/g to 4-$\log_{10}$ CFU/g. After that, APC declined again to 2-$\log_{10}$ CFU/g at 42 days of storage was observed. The total mean of both sample honeys was 3.58 $\log_{10}$

Table 1. Aerobic plate count: ($\log_{10}$ CFU/g) of *H. itama* and *G. thoracica* honeys at 6 weeks of storage

Storage Days	N	Mean ($\log_{10}$ CFU/g)	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
0 Day	2	4.68	0.75	0.53	-2.08	11.45	4.15	5.22
7 Days	2	5.17	0.08	0.06	4.41	5.93	5.11	5.23
14 Days	2	3.45	0.32	0.23	0.59	6.31	3.23	3.68
21 Days	2	2.00	0.00	0.00	2.00	2.00	2.00	2.00
28 Days	2	4.00	0.21	0.15	2.12	5.87	3.85	4.15
35 Days	2	3.78	0.42	0.30	-0.04	7.59	3.48	4.08
42 Days	2	2.00	0.00	0.00	2.00	2.00	2.00	2.00
Total	14	3.58	1.20	0.32	2.89	4.28	2.00	5.23

Dependent Variable: Pca Count with Mean ($\log_{10}$ CFU/g)

CFU/g. Both APC results in sample A and B were within the safe limit for microbiological count, which less than 5.00 $\log_{10}$ CFU/g, as stated by the Ministry of Health, Malaysia (FSANZ, 2016).

Yeast count in two types of Meliponini honeys

Identification and characterisation of yeast species have been reported by Barnett *et al.,* 1990, which based on morphological traits and physiological capabilities. The yeast counts of both samples were not significant differences ($p>0.05$) between *H. itama* and *G. thoracica.* The yeast count of both honeys was low in a fresh sample 10^4 CFU/g (Table 2). As the storage applied, both honeys indicated a number of yeast increased drastically from 10^4 to 10^6 CFU/g after 7 days of storage. At day 14 and 21 of storage days, the yeast count was decreased (10^2 CFU/g). However, during day 28, the yeast count increased to 10^4 CFU/g and then declined again to 10^3 CFU/g in day 35 and 42 days.

***Bacillus* sp. count in two types of Meliponini honeys**

The *Bacillus* sp. counts in both Meliponini honeys were not significant differences ($p>0.05$) indicating that both honey of *H. itama* and *G. thoracica* did not have much different in *Bacillus* sp. count (Table 3). However, the *Bacillus* sp. count in sample A (*H. itama*) had slightly higher count (2.93 $\log_{10}$ CFU/g) compared to sample B (*G. thoracica*) throughout 6 weeks of storage. There were only slightly different between both samples and the counts were low. The *Bacillus* sp. counts of both honeys was the highest at day 14. During 14 day of storage, the *Bacillus* sp. count increased drastically from 10^2 to 10^4 CFU/g. Then, the *Bacillus* sp. count declined to 10^2 CFU/g at day 28 and 35, respectively. The lowest count of *Bacillus* sp. was observed after 42 days of storage. The fluctuation in *Bacillus* sp. count occurred due to the microbial adaptation throughout storage.

Lactic acid bacteria (LAB) count in two types of Meliponini honeys

As lactic acid bacteria were monitored throughout storage, the results present were also different based on the media used. Based on Table 4, the LAB count in sample A (*H. itama*) was not significantly different ($p>0.05$) with sample B. However, the LAB count in sample B (*G. thoracica*) had higher count (6.55 $\log_{10}$ CFU/g) compared to sample A (*H. itama*) throughout 6 weeks of storage (Table 4).

Table 2. Yeast count: (Log_{10} CFU/g) of *H. itama* and *G. thoracica* honeys at 6 weeks of storage

Storage Days	N	Mean (Log_{10} CFU/g)	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
0 Day	2	4.13	0.01	0.01	4.00	4.25	4.12	4.14
7 Days	2	6.13	0.02	0.02	5.93	6.32	6.11	6.14
14 Days	2	3.84	2.27	1.61	-16.58	24.27	2.24	5.45
21 Days	2	3.58	2.24	1.58	-16.53	23.69	2.00	5.17
28 Days	2	4.54	0.01	0.01	4.45	4.64	4.54	4.55
35 Days	2	3.89	0.83	0.59	-3.58	11.35	3.30	4.48
42 Days	2	3.60	0.49	0.35	-0.82	8.01	3.25	3.95
Total	14	4.24	1.26	0.34	3.51	4.97	2.00	6.14

*Dependent Variable: Yeast Count with Mean ($\log_{10}$ CFU/g).

Table 3. *Bacillus* sp count: (Log_{10} CFU/g) of *H. itama* and *G. thoracica* honeys at 6 weeks of storage

Storage Days	N	Mean (Log_{10} CFU/g)	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
0 Day	2	2.00	0.00	0.00	2.00	2.00	2.00	2.00
7 Days	2	2.15	0.21	0.15	0.24	4.06	2.00	2.30
14 Days	2	3.78	0.90	0.64	-4.29	11.84	3.14	4.41
21 Days	2	2.15	0.21	0.15	0.24	4.06	2.00	2.30
28 Days	2	3.74	0.15	0.11	2.37	5.10	3.63	3.85
35 Days	2	3.71	0.23	0.17	1.61	5.80	3.54	3.87
42 Days	2	2.00	0.00	0.00	2.00	2.00	2.00	2.00
Total	14	2.79	0.90	0.24	2.27	3.31	2.00	4.41

*Dependent Variable: *Bacillus* sp. Count with Mean ($\log_{10}$ CFU/g).

Table 4. LAB count on MRS Agar: (Log_{10} CFU/g) of *H. itama* and *G. thoracica* honeys at 6 weeks of storage

Storage Days	N	Mean (Log_{10} CFU/g)	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
0 Day	2	8.82	0.19	0.13	7.13	10.50	8.69	8.95
7 Days	2	7.36	1.02	0.72	-1.82	16.54	6.64	8.09
14 Days	2	6.91	2.70	1.91	-17.36	31.18	5.00	8.82
21 Days	2	6.04	0.70	0.50	-0.28	12.36	5.54	6.54
28 Days	2	6.19	0.30	0.21	3.49	8.89	5.98	6.41
35 Days	2	5.01	0.42	0.30	1.19	8.82	4.71	5.31
42 Days	2	4.98	0.84	0.60	-2.58	12.54	4.39	5.58
Total	14	6.47	1.57	0.42	5.56	7.38	4.39	8.95

Dependent Variable: LAB count on MRS Agar with Mean (log_{10} CFU/g).

Table 5. LAB count on MRS Agar + 0.8% $CaCO_3$ (log_{10} CFU/g) of *H. itama* and *G. thoracica* honeys at 6 weeks of storage

Storage Days	N	Mean (Log_{10} CFU/g)	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
0 Day	2	9.53	0.51	0.36	4.99	14.07	9.17	9.89
7 Days	2	6.23	0.32	0.23	3.37	9.08	6.00	6.45
14 Days	2	6.93	2.72	1.93	-17.53	31.38	5.00	8.85
21 Days	2	5.69	0.42	0.30	1.94	9.43	5.39	5.98
28 Days	2	6.32	0.08	0.06	5.62	7.02	6.27	6.38
35 Days	2	5.32	0.08	0.06	4.55	6.08	5.26	5.38
42 Days	2	4.89	0.89	0.63	-3.15	12.92	4.26	5.52
Total	14	6.41	1.68	0.45	5.44	7.38	4.26	9.89

*Dependent Variable: LAB count on MRS Agar + 0.8% CaCO with Mean (log_{10} CFU/g).

Table 6. LAB count on MRS Agar + 1% glucose: (Log_{10} CFU/g) of *H. itama* and *G. thoracica* honeys at 6 weeks of storage

Storage Days	N	Mean (Log_{10} CFU/g)	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
0 Day	2	8.84	0.05	0.04	8.36	9.31	8.80	8.88
7 Days	2	6.94	0.74	0.52	0.33	13.55	6.42	7.46
14 Days	2	6.86	2.62	1.86	-16.72	30.43	5.00	8.71
21 Days	2	6.26	0.86	0.61	-1.46	13.98	5.65	6.87
28 Days	2	6.53	0.70	0.50	0.24	12.81	6.03	7.02
35 Days	2	4.14	1.61	1.14	-10.29	18.56	3.00	5.27
42 Days	2	4.98	0.61	0.43	-0.48	10.44	4.55	5.41
Total	14	6.36	1.73	0.46	5.36	7.36	3.00	8.88

*Dependent Variable: LAB count on MRS Agar + 1% glucose with Mean (Log_{10} CFU/g).

Table 5 shows substantial difference in LAB count on MRS agar with 0.8% $CaCO_3$ from both honey samples throughout storage days. The LAB count in sample A (*H. itama*) was not significantly different ($p>0.05$) with sample B (*G. thoracica*). However, the LAB count in sample A (*H. itama*) had higher count (6.62 log_{10} CFU/g) compared to sample B (*G. thoracica*) throughout 6 weeks of storage. The counts were significantly high and there were only slightly different on LAB counts between both samples.

Table 6 shows the LAB count on MRS agar with 1% glucose from both honey samples throughout storage days. As the results obtained, the LAB count on MRS agar with 1% glucose from sample A (*H. itama*) was not significantly different ($p>0.05$) with sample B (*G. thoracica*). However, the LAB count in sample A had lower count (6.17 log_{10} CFU/g)

compared to sample B (6.55 $\log_{10}$ CFU/g) throughout 6 weeks of storage. There were only slightly different between both samples and the bacteria population were high.

Table 6 shows the results of LAB count on MRS agar with 1% glucose in *H. itama* and *G. thoracica* honeys after 6 weeks of storage. Based on the results obtained, the LAB counts on MRS agar of both honeys were the highest at day 0 with 10^8 CFU/g while the lowest was the last day of storage (day 42) with 10^4 CFU/g was observed. Then, at day 7 the LAB count was decreased drastically from 10^8 to 10^6 CFU/g. Furthermore, the LAB counts were declined throughout storage days with some fluctuation between day 21 and 28.

Detection of *Clostridium* sp. in two types of Meliponini honeys

Based on the results, the obtained colony from anaerobic egg yolk agar were positive in both catalase and oxidase test. For *Clostridium* sp. they were sensitive bacteria that cannot exposed even in small amount of oxygen and should have negative results for catalase test. However, pure colony had negative results for both catalase and oxidase test which represented the actual results for *Clostridium* sp. characteristics. The detection of pathogenic bacteria *Clostridium* sp. in *H. itama* and *G. thoracica* honey were negative.

Determination of pH changes in two types of Meliponini honeys throughout ambient storage

The importance of acidic pH range in foods cannot be overemphasized because it prevents the honey samples from constant contamination by various species of micro-organisms and thus helps to ensure longer shelf life. Figure 1 shows the pH value of *H. itama* and *G. thoracica* honeys throughout 6-weeks of ambient storage. In this study, there were no significant differences at $p > 0.05$ between sample A and B except for day 7, 14, 28, and 42 there were a significant difference at $p<0.05$ between sample A and sample B.

DISCUSSION

Relationship between the presences of LAB microflora with other pathogenic bacteria

Quality assessment of microbiological analysis of Meliponini honey can be seen through the microflora counts throughout the storage days. In this study, LAB microflora were competitively grown with other pathogenic bacteria which were aerobic plate count, yeast count and *Bacillus* count. Results indicated that LAB count on MRS with 0.8% $CaCO_3$ was the highest when compared to MRS with 1% glucose and MRS alone. This result is in the agreement with previous study reported by Aween *et al.* 2012a, MRS with 0.8% $CaCO_3$ agar is the most suitable media for LAB isolation. In addition, calcium carbonate was used as an indicator for acid-producing strains as it dissolved when interacted with acid, then a clear zone was observed (Onda *et al.,* 2012).

The Aerobic Plate Count was lower in honey due to high number of LAB present in honey. According to Brudzynski and Kim (2011), there were reductions of antibacterial activity due to honey storage. This is because the growth of LAB were reduced throughout

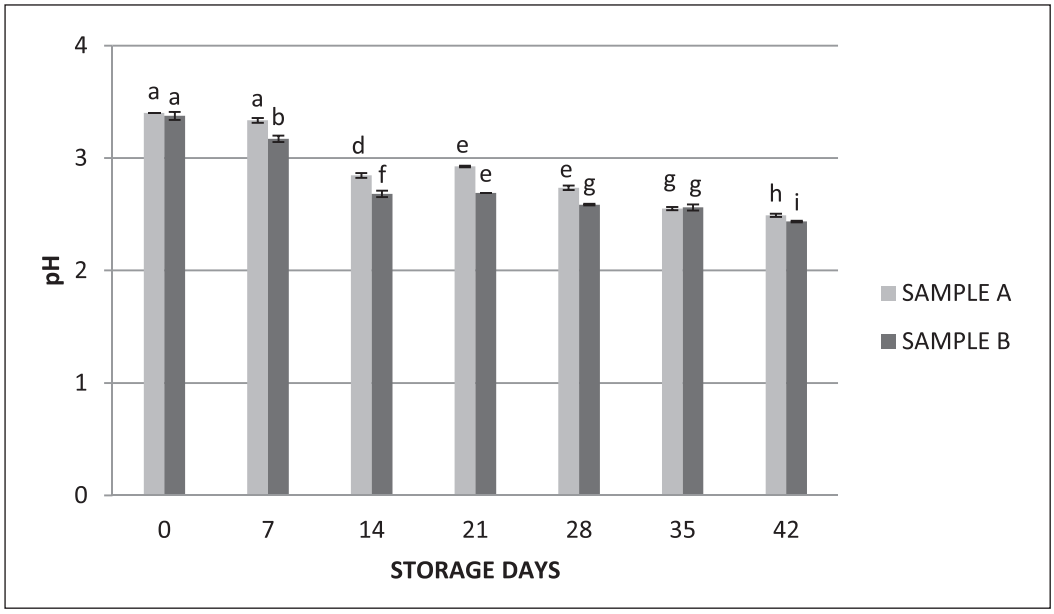

Fig. 1. The pH value of *H. itama* and *G. thoracica* honeys throughout 6-weeks ambient storage.
*Mean value with the different letter in the same day of storage days was significantly different at $p<0.05$.

storage. LAB has been known to have major roles in antimicrobial properties in honey. This study also shown that in three weeks of storage, LAB counts were high between 10^6 to 10^8 CFU/g, but then it decreased until 10^4 CFU/g at the last day of storage. In addition, at day 35 and 42 of storage, the LAB counts were low in both honey samples. Thus, it is advisable to consume Meliponini honey within one month of storage at ambient temperature after freshly harvested in order to get the highest population of LAB in stingless bee honeys.

The competition between yeast and LAB was observed where LAB counts were decreasing, while the yeast count were increased throughout storage. It is suggested that the main reason for competition is for sake of nutrients (carbohydrate source) for microbial metabolism. This kind of interaction between groups of bacteria is referred to 'antagonistic' interaction (Olofsson *et al.,* 2016). Antagonistic studies are generally directed towards food-spoilage and pathogenic microorganisms related to the host or product from which the lactic acid bacteria was isolated. The LAB counts increased again at day 14 while the yeast count decreased. The yeast was maintained in low count which 10^2 to 10^4 CFU/g until the last day of storage. The yeast count was low due to the low in pH value of samples (pH 2). The optimal pH range for yeast growth can vary from pH 4 to 6, depending on temperature, the presence of oxygen, and the strain of yeast (Neelakantam & Ronan, 2005).

pH changes in Meliponini honeys throughout 6-weeks of ambient storage

The pH values of both honey samples was low and declined gradually throughout storage days starting day 0 until day 42. Low pH in the acidic range may be an indication of good shelf life (Turhan *et al.,* 2008). Results from this study had shown the statistical significant of pH between both honeys samples were same with the previous study by Kacaniova *et al.* (2007), who had reported that the statistical different of measured pH values was variable in different groups of honey. Other studied by Crane (1990) also stated that the pH value was influenced by organic acids and by the concentration of mineral substances.

The mean pH of *H. itama* and *G. thoracica* samples analysed was ranged between pH 2.40–3.40 that make it possible that both honey samples may have a good antibacterial property. However, the reducing of pH value through storage might cause the decline of LAB growth due to acidic environment in honey. These results were supported by previous study, the optimal pH for LAB growth lies between pH 5 and 9 because lactic acid bacteria are neutrophils (Neelakantam and Ronan, 2005). So, LAB cannot grow under acidic environment.

CONCLUSION

The results obtained in this study indicated that there were relationship between the presence of LAB microflora and others pathogenic bacteria in honey during storage. Besides, the pH values were decreased in both honey samples throughout 6-weeks of storage. The pH ranged between 2.40–3.40 were low and suitable for inhibitor of pathogenic microbes since the most bacteria and mold grow optimum in the neutral pH (pH7). *Clostridium* sp. was absent in both honey samples indicating that both stingless bee honey were safe to be consumed.

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