CHAPTER 5

Long Term Storage Effects on Stingless Bee (Hymenoptera: Apidae: Meliponini) Honey

5.1 Abstract

The long term storage effects on stingless bee (*Tetragonula laeviceps-pagdeni*) honey from SE Asia (Thailand) were examined by using physicochemical parameters. Fresh stingless bee honey was stored at 4°C, 30°C and 45°C for 6 and 12 months. The results reveal the moisture, ash and electrical conductivity show only small levels of change over time and temperature storage. The total acidity increased when stored for 6 and 12 months. pH, diastase and HMF demonstrated statistically significant changes for both time and temperature storage. The carbohydrates (fructose, glucose and maltose) decreased during time and temperature storage but the changes were not statistically significant. Storage at the longest time period (12 months) and highest temperature (45°C) resulted in the greatest changes. Storage at 4°C for 12 months resulted in the least change and the honey was, by and large, unchanged from fresh honey.

Keywords: Honey storage, Physicochemical analysis, Meliponini, Stingless bee, *Tetragonula laeviceps-pagdeni*, South East Asia

5.2 Introduction

Honey from the western honey bee species *Apis mellifera* presently dominates the world trade market. Honey is however, produced by a large number of social insect species in the order Hymenoptera, including wasps, ants and most especially bees in the family Apidae (Burgett, 1974; Burgett & Young, 1974; Michener, 1974; Wilson, 1971). The consumption of honey pre-dates human history (Crane, 2013). It has been suggested that the first honeys incorporated into the human diet came from stingless bee (Apidae: Meliponini) in the neo-tropics of Central and South America (Vit *et al*., 2013) several millennia in the past.

According to Codex (2001) and IHC (2009) honey standards developed for the western honey bee, *A. mellifera*, (moisture, ash, electrical conductivity, pH, acidity, diastase, HMF and carbohydrates) are important parameters for determining honey quality. For stingless bee honey there are no published quality standards, although in a comprehensive analysis (Souza *et al*., 2006) recommendations have been put forward.

The physicochemistry of honey is best known for the western honey bee (Crane 2013; White *et al*., 1962; White *et al*., 1975; White, 1994). While generally recognized as a stable product and capable of long-term storage, the effects of time and temperature can markedly reduce honey quality (Castro-Vazquez *et al*., 2008; White, *et al*., 1962). From the view point of honey as a product in the world market, quality is the most frequently measured using several parameters including the presence of hydroxymethylfurfural (HMF) and the enzyme diastase, although the use of diastase as a metric for honey quality has been questioned (White, 1994). The presence of HMF is felt to be a reflection of heat exposure over longer-term storage (Ajlouni & Sujirapinyokul, 2010; Fallico *et al.,* 2004; White *et al*., 1964).

In a cosmopolitan sense, stingless bee honey production and consumption have historically been highly localized. From a commercial perspective, international trade in meliponine honey is miniscule relative to honey from *A. mellifera*. However an international commerce, albeit small, has developed for stingless bee honey (Chuttong *et al*., 2014; Ayala *et al*., 2013).

Thailand is known to possess 32 species of stingless bees (Rasmussen, 2008), of which *ca.* 5 species are managed both for the pollination benefit and the extraction of honey. For those stingless bee species that are involved in Thai meliponiculture, one species complex (*Tetragonula laeviceps-pagdeni*) is the most frequently encountered (Chuttong *et al*., 2014). While the physicochemistry of stingless bee honey is much less studied than the western honey bee, there is a developing literature, largely from stingless bee species of the neo-tropics, which addresses honey quality issues (Vit *et al*., 2013). One major objective of stingless bee honey research is to see the creation of national and international standards, which presently do not exist.

As stated by Menezes *et al*. (2013) there is a near absence of studies that analyze the changes in stingless bee honey over the course of time and is necessary for understanding changes in honey physicochemistry resulting from longer term storage. It is this specific issue we address with this research on the physicochemical changes occurring in stingless bee honey over time and at three temperature storage regimes. For our analyses we have chosen honey from one stingless bee species complex (*T. laeviceps-pagdeni*) as it presently dominates stingless bee honey production in Thailand.

5.3 Materials and Methods

5.3.1 Sample collection

Honey samples of the stingless bee species complex *Tetragonula laeviceps-pagdeni* were collected from stingless bee colonies in both natural and managed colonies from hives within the three Thai provinces of Chiang Mai, Chanthaburi and Trat. Sample collections took place during 2013. Honey storage pots were pierced with a sharp tool, and the honey was strained *via* gravity through fine cloth (gauze). Alternately, syringe extraction of individual and collective honey pots was done. Honey samples were immediately stored at 4°C in sealed glass jars kept in the dark. A total of 5 kg of honey was field collected from 50 stingless bee colonies from the provinces of Chiang Mai, Chanthaburi, and Trat. Individual samples represent honey pooled from several colonies in each of the 3 Thai provinces (n = 10; Chiang Mai 3, Chanthaburi 5, Trat 2).

5.3.2 Treatments

Each of the 10 honey samples was divided into seven 50 g. aliquots; one portion being devoted to immediate physicochemical analysis (*in natura*). The remaining 6 portions were assigned to three constant temperatures (4°, 30° and 45°C) and two storage times (6 and 12 months). Honey samples were held in incubators/environmental chambers maintained at the 3 temperatures (Sanyo Incubator MIR-153).

5.3.3 Analytical methods

The following honey parameters were examined: moisture (g/100g), ash (g/100g), electrical conductivity (ms/cm), pH, total acidity (meq/kg), diastase (°Gothe), hydroxymethylfurfural (mg/kg) and the individual carbohydrates fructose, glucose, maltose and sucrose (g/100g). Analytical methods follow those of AOAC.

1) Moisture

Honey moisture was determined by refractometry (AOAC method 919.38, 2006), using an Atago (Japan) model N-3E refractometer. All measurements were performed at 20 °C.

2) Ash content

Honey Ash content (AOAC method 920.181, 2006) was measured by placing a crucible in a 100 °C oven for one hour. After cooling it was weighed. Aliquots of 5 g of honey were placed into the crucible and then incinerated in Muffle furnace at 500°C to constant weight (overnight) and then reweighed. Ash percentage was calculated.

3) Electrical conductivity (EC)

Honey electrical conductivity was determined according to International Honey Commission, 2009 by measured in a 20% (w/v) solution of honey in distilled water using a Cyberscan waterproof (Singapore) model PC300 Series digital conductometer.

4) pH and total acidity

Honey pH and total acidity were measured according to AOAC method 962.19, 2006. Total acidity was determined by the titrimetric method. Ten g of honey was dissolved in 75 ml distilled water, and this solution was titrated with 0.05 M NaOH solution until the pH reached 8.5. Ten ml of 0.05 M NaOH was added immediately, and back-titrated with 0.05 M HCl solution until the pH reached 8.3 (lactone acidity) to determine the acidity. A Cyberscan waterproof (Singapore) model PC300 Series digital pH meter was used to take the pH measurements.

5) Diastase activity

Honey diastase activity was determined according to AOAC method 958.09, 2006 by placing 5 g of honey into a 20 ml beaker and diluting with 10 ml distilled water and 2.5 ml of acetate buffer (1.59 M, pH 5.3). It was transferred to a 25 ml volumetric flask containing 1.5 ml of 0.5 M NaCl solution. Ten ml of honey solution were incubated in a thermostatic bath at 40°C along with a second flask containing 100 ml of 1% (w/v) starch solution. After 5 min, 5 ml of starch solution was added to the honey solution. After 5 min 1 ml of the mixture was mixed with 10 ml of 0.0007 M diluted iodine solution, and measured at 660 nm in a Shimadzu UV-1601 UV/VIS spectrophotometer, compared with a water blank. A plot of absorbance against time was used to determine the time at which the specified absorbance of 0.235 was reach.

Diastase is the enzyme responsible for converting starch to dextrins and sugars. It is added to ripening honey by the bees. The diastase number expresses the diastase activity as the number of ml of a 1% starch solution hydrolyzed by the enzyme in 1 g of honey in 1 hour at 40°C. The results are expressed in Gothe degrees. The use of diastase as a honey quality indicator has been questioned (White, 1994).

6) Hydroxymethylfurfural (HMF)

Honey HMF contents were determined according to AOAC method 980.23, 2006 by high performance liquid chromatography (HPLC) coupled to UV spectrometry. A 5% (w/v) solution of honey in distilled water and filtered through 0.45 µm filter paper and injected into HPLC system (Shimadzu, Kyoto, Japan), which was equipped with a LC-10AD VP pump, a 7125 Rheodyne injector, SCL 10 AVP system controller, a diode array detector SPD-M10A, and class VP controller software. Isocratic elution was performed on a reversed-phase ultra C18 ODS (5 µm, 250 x 4.6 mm) column (Restex, Bellefonte, Pennsylvania, USA), using as mobile phase methanol-water (10:90, v/v) at a flow rate of 1.2 mL/min and the detection, at 280 nm (Mendes et al., 1998).

7) Sugars

Honey sugars contents (sucrose, glucose, fructose, and maltose) were determined according to AOAC method 977.20, 2006 were determined by high performance liquid chromatography (HPLC) coupled to refractive index detector (RID). A 5% (w/v) solution of honey in distilled water and filtered through 0.45 µm filter paper and injected into HPLC system (Shimadzu, Kyoto, Japan), which was equipped with a LC-10AD pump, CBM-10A system controller, a RID-10A refractive index detector coupled to a computer with class LC10 controller software. For the determination of sugars an Inersil NH_2 column (5 µm, 250 x 4.6 mm) (GL science Inc., Japan) mobile phase with HPLC acetonitrile/water (72:25) was used at a flow rate of 1 ml/min, with an oven temperature of 40°C (Mendes et al., 1998).

5.3.4 Statistical analysis.

Comparative data were subjected to the Duncan's multiple-range test (MRT) (Statistica 6.0) to determine the significance between time and temperature treatments.

5.4 Results

The physiochemical and organoleptic qualities of honey are predominated by the nectar resources gathered by colony foragers (Crane 1975). Additional factors exerting influence are biogeographic, climate and annual seasonality. Angiosperms produce nectar for the function of attracting pollinators and plant species have been shown exhibit qualitative differences in nectar constituents most especially the carbohydrates (Crane, 1975). Therefore a given honey from a stingless bee species represents a chemical profile uniquely representing the botanical resources, climate and time of year when the bees elaborated the nectars to honey within the confines of the colony environment. No single honey analysis can be said to completely represent the variability in chemical complexity.

5.4.1 *T. laeviceps-pagdeni honey in natura*

Table 5.1 and 5.2 provide the summary of the baseline analysis of fresh *T. laeviceps-pagdeni* honey (*in natura*) for all parameters measured. These observations are in general agreement with previous descriptions of stingless bee honey from both SE Asia (Suntiparapop *et al*., 2012; Sawattham *et al*., 2009) and the neo-tropics (Vit *et al*., 2013). Relative to established national and international standards set for the western honey bee *A. mellifera*, honey from *T. laeviceps-pagdeni* is higher in moisture, ash, acidity and lower in diastase and reducing sugars. The disaccharide maltose is present in higher concentrations, frequently being the dominant carbohydrate.

Table 5.1 Physicochemical analyses of fresh *Tetragonula laeviceps-pagdeni* honey

Location	Moisture (g/100g)	Ash (g/100g)	EC (ms/cm)	pH	Total acidity (mEq/kg)	Diastase (°Gothe)	HMF (mg/kg)
CM1	23.0	0.98	2.1	3.3	118.0	4.8	56.2
CM2	26.1	0.64	1.5	3.5	109.0	1.1	5.9
CM3	21.0	0.69	1.3	3.9	105.0	1.7	36.0
CH1	25.3	0.28	0.6	3.9	117.0	1.5	3.6
CH2	25.9	0.31	0.7	3.6	110.0	ND	27.0
CH3	25.8	0.51	1.3	4.0	97.0	ND	66.0
CH4	26.8	0.16	0.8	3.4	115.5	ND	2.2
CH5	23.7	0.26	0.6	3.9	110.0	0.8	6.7
TR1	24.9	0.26	0.7	4.1	91.0	4.6	68.8
TR2	24.5	0.28	0.7	3.6	78.5	ND	58.0
Average ± SD	24.7± 1.7	0.44 ± 0.26	1.0 ± 0.5	3.7 ± 0.3	105.1 ± 12.7	2.4 ± 1.8	33.0 ± 27.5

CM = Chiang Mai province (3 samples), CH = Chanthaburi province (5 samples),

TR = Trat province (2 samples), ND = Not detected

Table 5.2 Sugar contents of fresh *Tetragonula laeviceps-pagdeni* honey

Location	Fructose (g/100g)	Glucose (g/100g)	Reducing sugar (g/100g)	Maltose (g/100g)	Sucrose (g/100g)	Total Sugar (g/100g)
CM1	15.3	11.2	26.5	40.6	ND	67.0
CM2	13.0	9.3	22.3	44.6	ND	66.9
CM3	12.2	6.8	19.0	53.3	ND	72.3
CH1	22.1	19.0	41.1	32.1	1.5	74.7
CH2	24.0	21.2	45.3	25.8	1.3	72.3
CH3	29.1	27.1	56.2	11.7	0.7	68.6
CH4	22.2	21.7	43.8	29.1	ND	73.0
CH5	23.4	22.1	45.5	26.3	0.3	72.1
TR1	22.2	19.3	41.5	29.9	ND	71.4
TR2	18.0	16.5	34.5	39.8	ND	74.3
Average ± SD	20.2 ± 5.4	17.4 ± 6.4	37.6 ± 11.8	33.3 ± 11.7	1.0 ± 0.5	71.3 ± 2.8

CM = Chiang Mai province (3 samples), CH = Chanthaburi province (5 samples),

TR = Trat province (2 samples), ND = Not detected

5.4.2 Treatment Effects

Table 5.3 Treatment effects of time and temperature on the physicochemical of *Tetragonula laeviceps-pagdeni* honey

Storage time (months)	Storage temperature (°C)	Moisture (g/100g)	SD	Ash (g/100g)	SD	EC (ms/cm)	SD	pH	SD	Total acidity (mEq/kg)	SD	Diastase (°Gothe)	SD	HMF (mg/kg)	SD
	Fresh	24.7	1.7	0.44	0.3	1.04	0.5	3.7a	0.3	105.1	12.7	2.4a	1.8	33.0c	17.5
6	4	24.8	2.0	0.43	0.3	1.19	0.6	3.6ab	0.2	110.3	45.3	2.3a	1.7	35.1c	30.4
	30	25.6	2.3	0.42	0.3	0.20	0.6	3.6ab	0.2	126.6	54.5	1.3ab	1.4	212.2c	87.0
	45	25.4	1.9	0.43	0.3	1.16	0.6	3.5ab	0.2	146.1	54.3	0.4b	0.4	3,881.0b	1,023.7
12	4	24.7	1.9	0.42	0.3	0.21	0.6	3.6ab	0.2	117.3	36.6	2.2a	1.9	35.1c	30.7
	30	25.5	1.9	0.41	0.3	1.21	0.6	3.5ab	0.1	122.3	45.3	0.5b	0.3	411.3c	166.4
	45	25.4	1.7	0.39	0.2	1.16	0.5	3.4b	0.1	147.2	53.6	0.4b	0.5	5,667.5a	1,627.3
Storage time		NS		NS		NS		<0.01		<0.05		<0.05		<0.01	
Storage temperature		NS		NS		NS		NS		NS		<0.05		<0.01	
Time x temperature		NS		NS		NS		<0.05		NS		<0.05		<0.01	

Column values that do not share a common letter are significant (Duncan's Multiple Range Test). Columns without letters display no significant difference

Table 5.4 Treatment effects of time and temperature on the carbohydrates of *Tetragonula laeviceps-pagdeni* honey

Storage time (months)	Storage temperature (°C)	Fructose (g/100g)	SD	Glucose (g/100g)	SD	Reducing sugar (g/100g)	SD	Maltose (g/100g)	SD
	Fresh	20.2	5.4	17.4	6.4	37.6	11.2	33.3	11.7
6	4	19.6	5.4	16.6	6.7	36.2	12.0	31.4	10.7
	30	19.3	4.8	16.5	7.1	35.8	11.9	28.8	9.2
	45	18.7	4.6	14.9	7.1	33.6	11.5	25.6	7.2
12	4	18.2	4.9	15.1	6.3	33.3	11.1	31.1	10.8
	30	17.8	4.0	14.7	6.1	32.5	10.1	27.6	9.1
	45	17.7	3.8	14.2	6.3	31.9	10.1	26.8	7.9
Storage time		NS		NS		NS		NS	
Storage temperature		NS		NS		NS		NS	
Time x temperature		NS		NS		NS		NS	

For the parameters of moisture, ash, electrical conductivity (Table 5.3 and Figure 1) and total acidity (Table 5.3 and Figure 2b) the effects of time x temperature storage reveal no statistically significant differences.

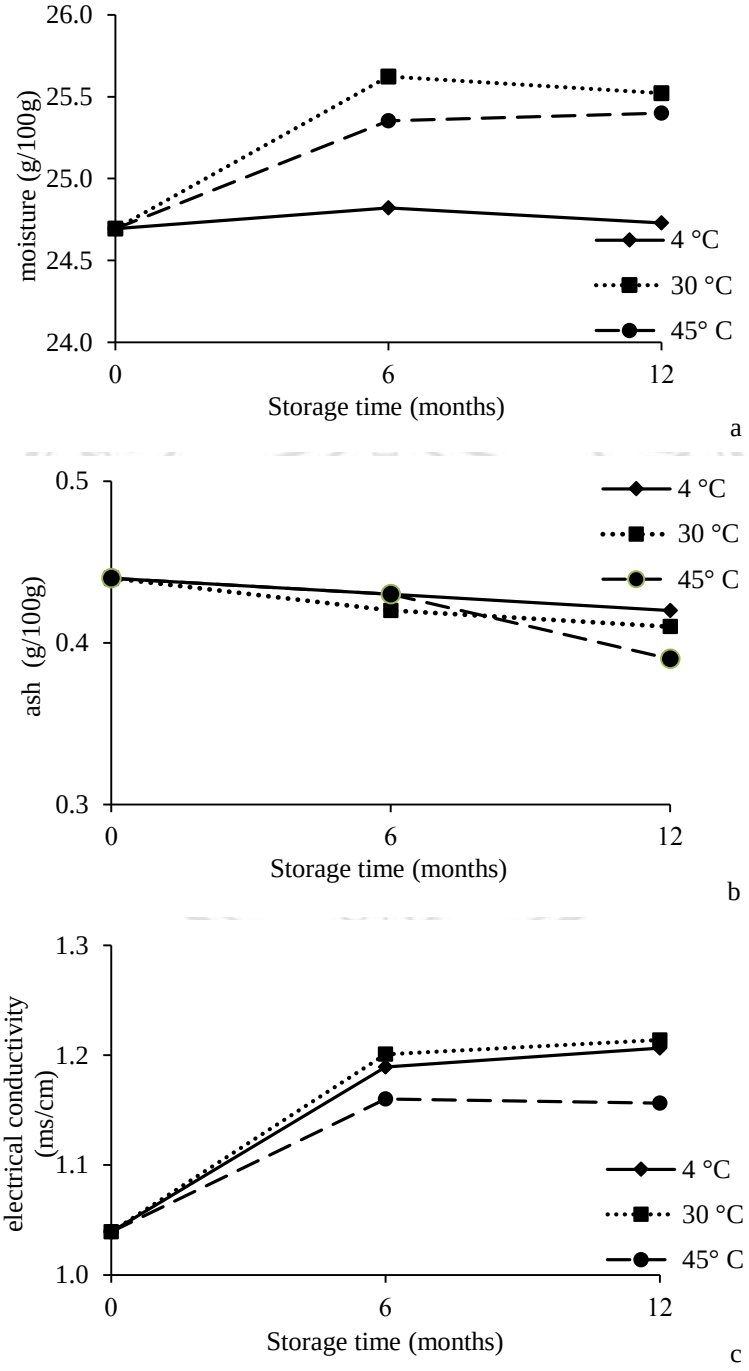

Figure 5.1 Changes in moisture, ash and electrical conductivity content of *Tetragonula laeviceps-pagdeni* honey during the time and temperature storage

For pH, significance is shown by the effect of time ($p < 0.01$) and time x temperature ($p < 0.05$) (Table 5.3 and Figure 2a). Total acidity is shown by the effect of time only ($p < 0.05$) (Table 5.3 and Figure 2b).

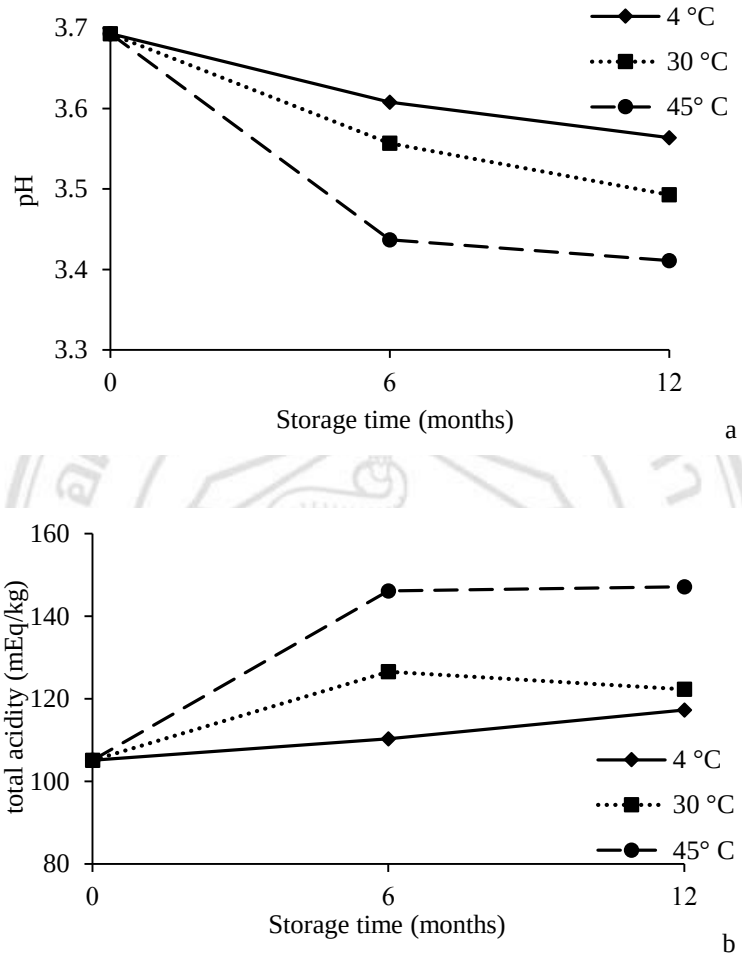

Figure 5.2 Changes in pH and total acidity content of *Tetragonula laeviceps-pagdeni* honey during the time and temperature storage

Diastase displays statistical significance by the effect of both time and temperature ($p < 0.05$) (Table 5.3 and Figure 3a). HMF was the measure that displayed the most dramatic effects of time and temperature and showed a high degree of statistical significance ($p < 0.01$) for both time and temperature. The HMF level after 12 months of storage at 45°C was amplified from the fresh honey HMF average by a factor of 171 (Table 5.3 and Figure 3b). From the analyses of fructose, glucose, and maltose, the effects of temperature and storage time revealed no statistically significant changes (Table 5.4 and Figure 3). Sucrose was detected only in the fresh honey samples at a low level (0.95 mg/kg) and was undetected following all time and temperature treatments.

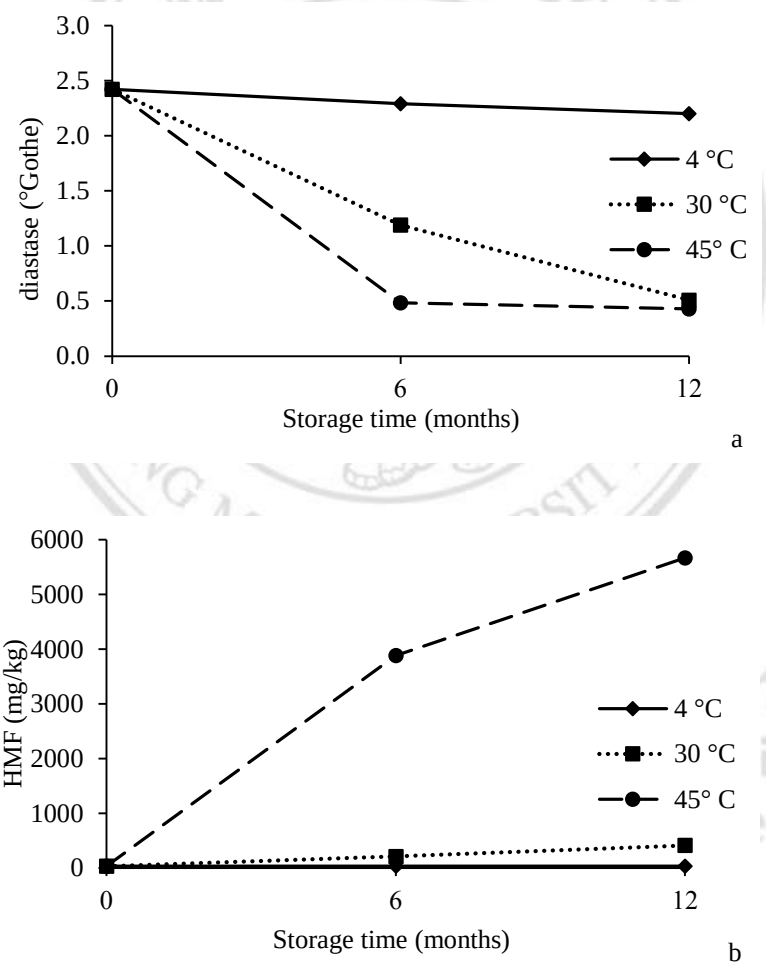

Figure 5.3 Changes in diastase and HMF content of *Tetragonula laeviceps-pagdeni* honey during the time and temperature storage

From the analyses of fructose and glucose the effects of temperature and storage time revealed no statistically significant changes (Table 5.4 and Figure 4).

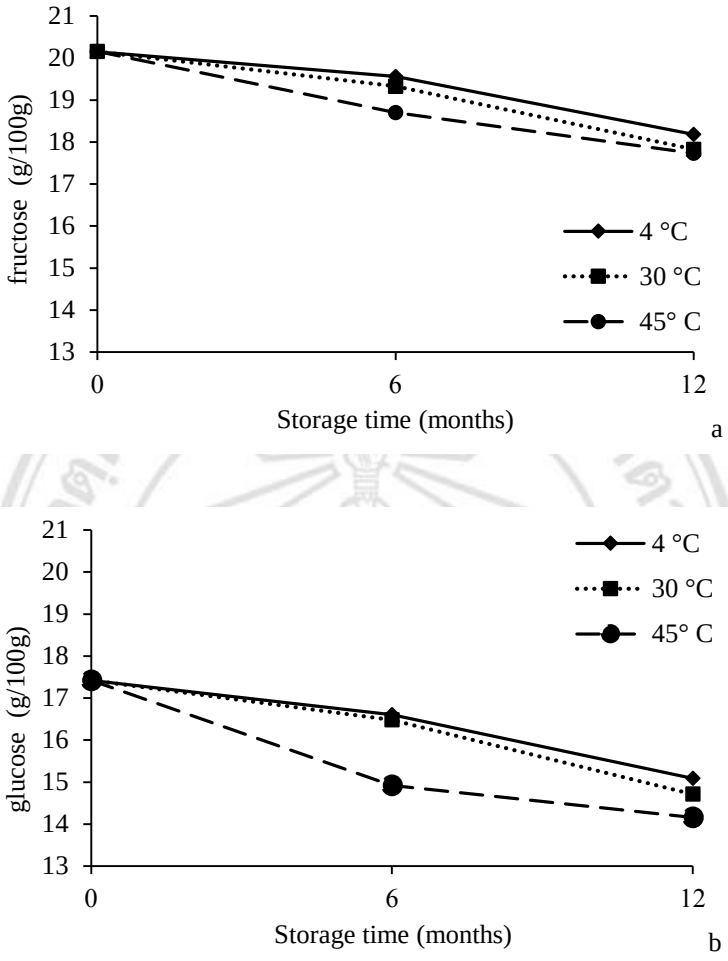

Figure 5.4 Changes in fructose and glucose content of *Tetragonula laeviceps-pagdeni* honey during the time and temperature storage

From the analyses of maltose, the effects of temperature and storage time revealed no statistically significant changes (Table 5.4 and Figure 5). Sucrose was detected only in the fresh honey samples at a low level (0.95 mg/kg) and was undetected following all time and temperature treatments.

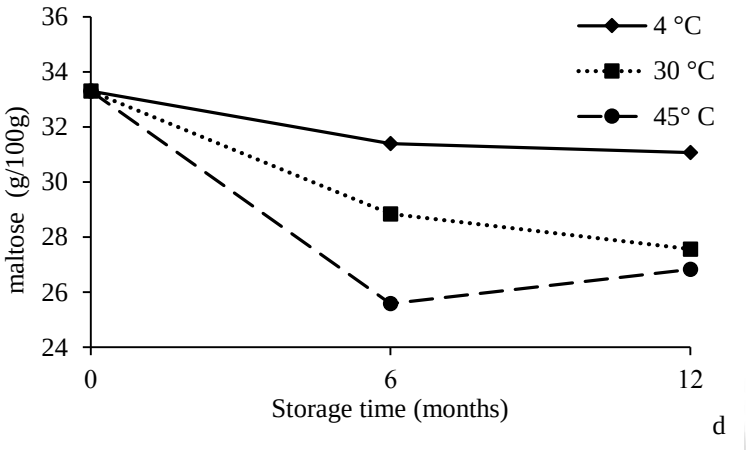

Figure 5.5 Changes in maltose content of *Tetragonula laeviceps-pagdeni* honey during the time and temperature storage

5.5 Discussion

5.5.1 *T. laeviceps-pagdeni* honey *in natura*

Our observations are in general agreement with previous descriptions of stingless bee honey from both SE Asia (Suntiparapop *et al.*, 2012; Sawattham *et al*., 2009) and the neo-tropics (Vit *et al*., 2013).

The value of the moisture content to the physical properties of honey is related to shelf life (Bogdanov *et al.*, 2004; Oddo & Piro, 2004). The international standard of moisture content in *A. mellifera* honey is not more than 20 g/100 g (Codex, 2001). For stingless bee honey, a suggested moisture standard is 25 g/100g (Souza *et al*., 2006). Stingless bee honey is known to possess higher levels of moisture relative to *A. mellifera* honey (Suntiparapop *et al.*, 2012; Vit *et al.*, 2013). When using the western honey bee comparator our result for fresh *T. laeviceps-pagdeni* honey is characterized

as higher in moisture (24.7 ± 1.7 g/100g). However, within the recommended standard for stingless bee honey (Souza *et al*., 2006).

Ash and electrical conductivity (EC) are parameters related to the mineral content in honey (Bogdanov *et al*., 2004). EC is correlated to the ash and acid content of honey. With increases in ash and acidity there is an expected corresponding increase in EC (Vorwohl, 1964). The results for ash content from our analysis of fresh *T. laeviceps-pagdeni* honey (0.44 ± 0.26 g/100g) reveal a higher level than A. mellifera honey (White *et al*., 1962) and higher than the ash content previously reported for *T. laeviceps* honey (Suntiparapop *et al*., 2012) from Thailand and other stingless bee species, *i.e., Melipona favosa* (Vit, 2013) from Venezuela and *Tetragonisca angustula* (Fuenmayor *et al*., 2013) from Columbia. The result for electrical conductivity of the fresh stingless bee honey (1.0 ± 0.5 ms/cm) is higher than that reported by Suntiparapop *et al.* (2012); Guerrini *et al.* (2009) for stingless bee honeys from Thailand and Ecuador respectively.

Honey is an acidic medium with a pH range between 3.5 and 5.5. Both flavor and antimicrobial properties of honey are influenced by the presence of organic acids in honey (Bogdanov *et al*., 2004). In our finding pH was (3.7 ± 0.3) similar to the report of Suntiparapop *et al*. (2012) for *T. laeviceps* honey but slightly lower than reported by Vit *et al.* (2013) for neo-tropical stingless bee honeys. Our samples, derived from 50 separate stingless bee colonies, resulted in an average acidity for fresh honey of 105.1 ± 12.7 mEq/kg with a range of 78.5 to 118.0 mEq/kg which is higher than reported for *T. laeviceps* honey (Suntiparapop *et al.,* 2012) and higher than the recognized standards for *A. mellifera* honey.

Diastase levels are thought to be an indicator of overheating (Bogdanov *et al*., 1997). Diastase levels in *A. mellifera* honey exhibit large variation but the standard is not less than 8 °Gothe (Codex, 2001). White *et al.* (1962) reported a range for *A. mellifera* honey from 3.1 to 22.2 °Gothe. For stingless bee honey diastase also varies with a range of 2.4 to 21.3 °Gothe and 0.9 to 23.0 °Gothe from the reports of Vit *et al.* (2013); Souza *et al.* (2006) respectively. Our observations of diastase in fresh *T. laeviceps-pagdeni* honey showed a range of not detected to 4.8 °Gothe; when present the average diastase was (2.4 ± 1.8 °Gothe). We did not detect diastase in 4 of our 10 honey samples.

Hydroxymethylfurfural (HMF) is the major quality factor used to evaluate the heating history of honey. HMF is thought not to be present in fresh *A. mellifera* honey as stored in the colony (Bogdanov *et al*., 1997; White, 1994). The Codex (2001) standard for *A. mellifera* honey specifies that the HMF content shall not be more than 40 mg/kg with the exception of honeys originating from tropical regions, not more than 80 mg/kg. For our fresh *T. laeviceps-pagdeni* honey the HMF averaged 33.0 ± 27.5 mg/kg with a range of 2.2 to 68.8 mg/kg which is higher than reported for *T. laeviceps* honey from Thailand Suntiparapop *et al*. (2012) and *Melipona favosa* honey from Venezuela (Vit, 2013).

Carbohydrates are the major component of honey. The Codex (2001) standard for *A. mellifer*a honey sets a criterion for reducing sugars (fructose and glucose) of ≥ 60g/100g. For stingless bee honey Vit *et al*. (2013) reported a range of 29.7 to 77.8 g/100g of reducing sugar from stingless bee honeys of the neo-tropics. Our observations of fresh *T. laeviceps-pagdeni* honey show it to be lower in reducing sugars with an average fructose content of 20.2 ± 5.4 g/100g with a range of 12.2 to 29.1 g/100g and the glucose average being 17.4 ± 6.4 g/100g with a range of 6.8 to 27.1 g/100g. These data are lower than those reported for *T. laeviceps* honey (Suntiparapop *et al.*, 2012) and for *A. mellifera* honey (White *et al*., 1962). Our findings for all 10 *T. laeviceps-pagdeni* honey samples presented values below the standard of *A. mellifera* honey. Our honey samples exhibit much higher levels of maltose which averaged 33.3 ± 11.7 g/100g with a range of 11.7 to 53.3 g/100g than those reported for *A. mellifera* honeys, or from *T. carbonaria* from Australia (20.3 ± 2.9 g/100g) (Oddo *et al*., 2008). For stingless bee honeys from the neo-tropics, maltose is reported to range from 2.5 to 32.2 g/100g (Vit *et al*., 2013). Our result shows a lower level of sucrose when compared to Suntiparapop *et al.* (2012). When present, sucrose averaged 1.0 ± 0.5 g/100g and ranged from not detected (6 of 10 honey samples) to 1.5 g/100g.

5.5.2 Treatment Effects

In general, the quality of honey decreases with time and temperature storage. These conditions can impose an effect on the physicochemical and organoleptic characteristics of honey (Castro-Vazquez *et al.*, 2008).

1) Moisture

For both temperature and time effects moisture displayed no statistical significance. When stored at 4°C for 6 and 12 months moisture was stable. When stored at 30 °C and 45 °C for 6 months there was a very small incremental trend upward (Figure 1a). Our results exhibit a similar moisture trend to that reported by Castro-Vazquez *et al.* (2008) for *A. mellifera* honey from Spain.

2) Ash and electrical conductivity (EC)

In our trials, ash levels increased under all conditions but were so slight that no significant differences were shown for time or temperature (Table 5.3 and Figure 1b). EC is influenced by mineral and acid content of honey, *i.e.,* EC increases with increasing ash and acidity (Codex, 1993). In stored *A. mellifera* honey the EC is frequently higher when compared to fresh honey (Castro-Vazquez *et al.,* 2008). The electrical conductivity revealed a small upward trend over our treatment times and temperatures (Table 5.3 and Figure 1c), however no statistical significance was shown for either time or temperature, which is dissimilar to reports for EC from *A. mellifera* honey (Castro-Vazquez *et al.*, 2008).

3) pH and acidity

Under our experimental treatments pH showed a significant downward trend with increasing time (Table 5.3 and Figure 2a) which is in contrast to reports for *A. mellifera* honey during storage (Castro-Vazquez *et al*., 2008; Gulati & Kumari, 2005; Moreira, *et al*., 2007). Stingless bee honey is characterized as being more acidic when compared to honey from the western honey bee. While the acidity did increase with time and temperature, it was statistically significant for time but not temperature (Table 5.3 and Figure 2b). Our findings are in agreement with those of

Castro-Vazquez *et al*. (2008) who show a significant increase in acidity over time for *A. mellifera* honey.

4) Diastase activity

Storage and heating are known to affect the diastase activity in honey (Bogdanov *et al*., 1997). Our results reveal a significant downward trend of diastase during time and temperature storage which is not similar to the reports of Castro-Vazquez *et al*. (2008); Sahinler & Gul (2005) (Table 5.3 and Figure 2c). Diastase in our honey samples stored at 4°C was slightly decreased at 6 and 12 months and averaged 2.3 ± 1.7 °Gothe and 2.0 ± 1.9 °Gothe respectively. The diastase of honey stored at 30 °C for 6 and 12 months also decreased continuously. The largest change in diastase was honey stored at 45°C for 12 months (0.4 ± 0.5 °Gothe). Diastase when stored at 4°C for 6 and 12 months decreased by 6% and 16% relative to fresh honey. At 30°C and 45°C of storage diastase at 6 and 12 months was diminished by 50% and 78%; 80% and 82% respectively.

5) Hydroxymethylfurfural (HMF)

In honey HMF is produced from simple sugars, especially fructose, by acidification over time (Bogdanov *et al*., 1997; White, 1994). HMF is influenced by time, temperature storage and pH (White *et al*., 1962). Previous reports of HMF for presumed fresh stingless bee honeys from South America give a range of not detected to 25 mg/kg (Vit *et al*., 2013, appendix 1) and not detected to 78.5 mg/kg (Souza *et al*., 2006, Table 5.3).

In our observations HMF was the parameter most affected by time and temperature treatments (Figure 2d). As fresh, *T. laeviceps-pagdeni* honey HMF measured (33.0 ± 27.5) and following time and temperature treatments, rose to a high of 5,667.5 ± 1627.3 mg/kg after 12 months of storage at 45°C. However after 12 months at 4°C, the HMF (35.1 ± 30.7 mg/kg) was essentially identical to the HMF level of fresh honey (Table 5.3 and Figure 2d). Our results correspond to those of Castro-Vazquez *et al*. (2008) in their study of *A. mellifera* honey where their storage time and temperatures were similar to ours; however their reported HMF increase at their longest storage (12 mo.) and highest temperature (40°C) was markedly lower from our results. The results

differ from those of Biluca *et al*. (2014) who found HMF to be absent from fresh stingless bee honey (species not given) from Brazil and was generated only under a condition of 75°C for 24 hours. We assume that the higher total acidity of *T. laeviceps-pagdeni* fresh honey would be a significant influence in the dramatic HMF increase we observed over increasing time and temperature.

6) Carbohydrates

While displaying no statistical significance, the monosaccharides (fructose and glucose) and the disaccharide maltose did experience a downward trend over time (Figure 3) which was more pronounced with an increase in temperature. Stingless bee honey stored at 45°C exhibited the largest, albeit small reductions, in both reducing sugars and maltose. These findings for stingless bee honey are contrary to the report of White *et al*. (1961) which showed prolonged storage to increase invert sugars (fructose and glucose).

5.6 Conclusions

The combined effects of time and storage temperature were statistically significant for pH, diastase and HMF. A statistical significance was demonstrated for the effect of time alone on total acidity.

The pH decrease over time was statistically significant and this is in disagreement with the reports of Castro-Vazquez *et al*. (2008); Moreira *et al*. (2007) who found the pH of *A. mellifera* honey to be very stable over several regimes of time and temperature storage. Our stingless bee honey also demonstrated significance in the rise in total acidity related to storage time which would be a primary causation for the observed decrease in pH. This agrees with the observations of Castro-Vazquez *et al*. (2008) who demonstrated increasing total acidity but not a corresponding to stable in the pH of the *A. mellifera* honey they examined.

The parameters of moisture, ash and EC demonstrated no statistical significance for either storage time or storage temperature. This result is similar to the reports of Castro- Vazquez *et al.* (2008); Moreira *et al.* (2007) for *A. mellifera* honey which displayed stable moisture levels over time and temperature storage. EC levels for *T. laeviceps-pagdeni* honey increased slightly over storage time which is not similar to the report of Castro- Vazquez *et al*. (2008).

Stingless bee honey carbohydrates were little affected by time and temperature, although the monosaccharide reducing sugars and the disaccharide maltose all experienced downward trends. Quantitatively maltose was the dominant sugar. Fructose and glucose were lower than previous reports of neo-tropical stingless bee honey and unchanged with time and temperature treatment.

Our overall findings support the use of HMF as a good indicator of storage and temperature effects. From the results stingless bee honey should be stored at low temperature (4°C) which honey maintains many of its properties during 12 months. However, the changes of their composition make it recommendable to not extend the storage time for preserving the characteristics of fresh honey.