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Physicochemical profiles of stingless bee (Apidae: Meliponini) honey from South East Asia (Thailand)

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ABSTRACT

This study examines the physicochemical properties of stingless bee honey from SE Asia (Thailand). Twenty-eight stingless bee honey samples, from 11 stingless bee species, were examined. Results reveal an average color (67 ± 19 mm Pfund), moisture (31 ± 5.4 g/100 g), ash (0.531 ± 0.632 g/100 g), electrical conductivity (1.1 ± 0.780 ms/cm), pH of (3.6 ± 0.198), total acidity (164 ± 162 meq/kg), diastase activity (1.5 ± 1.6 °Gothe) and hydroxymethylfurfural (8.7 ± 12 mg/kg). The carbohydrate profile is: total sugar (51 ± 21 g/100 g), fructose (17 ± 9.7 g/100 g), glucose (14 ± 8.6 g/100 g), maltose (41 ± 15 g/100 g) and sucrose (1.2 ± 2.7 g/100 g). These findings are not dissimilar to those reported for stingless bee honeys from the neo-tropics. When compared with the *Apis mellifera* standard, stingless bee honey is characterized as possessing higher moisture content, acidity, ash and HMF but a lower level of total sugars.

1. Introduction

Stingless bees are highly eusocial insects. Stingless bees are pan-equatorial and can be found in most tropical and subtropical regions of the world e.g., Australia, Africa, Southeast Asia, meso-America and South America. They belong to the order Hymenoptera and have been placed in one of four tribes in the family Apidae (Meliponini). Worldwide there are over 500 described species in 32 genera with perhaps more than 100 new species to be characterized (Michener, 2000; Michener, 2013). Rasmussen (2008) has listed 89 species in 15 genera from the Indo/Australian region. Specifically for Thailand, 32 species in 10 genera have been described. As highly eusocial insects, stingless bee colonies can contain from as few as a hundred to more than a hundred thousand bees (Michener, 2013). As social bees they collect, store and chemically modify floral nectars which results in the production of honey, a natural product long sought after by humans for its organoleptic and putative medicinal properties. Stingless bees produce and store much less honey on a per hive basis when compared to the western honey bee *Apis mellifera*, the world leader in honey production. None-the-less, humans have exploited honey from stingless bee species throughout their tropical range for millennia.

Stingless bee beekeeping is known as meliponiculture. The utilization of stingless bees by humans is an ancient undertaking albeit not overly well documented in the archeological record (Ayala, Gonzalez, & Engel, 2013; Crane, 1992; Rosales, 2013). Meliponiculture in Thailand presently could be characterized as in an incipient phase with the number of stingless bee beekeepers and 'managed' stingless bee colonies increasing (Chuttong, Chanbang, & Burgett, 2014). Currently, the epicenter of stingless beekeeping in Thailand is the Southeast region centering on the provinces of Chanthaburi and Trat. The stingless bee species most commonly managed for culture and honey harvesting are members of the *Tetragonula laeviceps-pagdeni* complex, but also 'managed' are *T. testaceitarsis, T. fuscobalteata, Lepidotrigona flavibasis and L. doipaensis. T. laeviceps-pagdeni* is the most widely distributed species. It is found throughout Thailand. The genus *Tetragonula* is suspected to contain numerous cryptic species and a more complete understanding of the taxonomy of this group awaits future molecular genetic diagnosis. Numerous *Tetragonula* species are very opportunistic in their choices of nesting cavities and for this reason are among the most common taxon for meliponiculture owing to their willingness to accept human-made domiciles (hives). Production of stingless bee honey at any level, when compared to the production of *A. mellifera* honey, is miniscule,

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e.g. Chuttong et al. (2014) estimate the total national production for Thailand to be 2.5–3 metric tons. The limited production, especially as an international commodity, results in a near complete lack of honey quality standards especially at an international level. Due to insufficient knowledge about the product, stingless bee honey is not included in international standards for honey (Codex, 2001) and it is not regulated by food control authorities. For stingless bee honey, there are no published quality control declarations for consumers. The aim of the International Honey Commission (IHC) is the establishment of quality standards of bee products other than western honey bee (*A. mellifera*), including stingless bee honey. Brazil and Venezuela are attempting to establish national standards for stingless bee honey following the guidelines of international standards of the Codex Alimentarius Commission (Souza et al., 2006).

While a body of literature exists that describes many physicochemical properties of stingless bee honey, these works have to a large degree concentrated on stingless bee species of the neo-tropics in Central and South America. Previous works on the physicochemical characteristics of stingless bee honey have been done in several countries in South America. Vit, Bogdanov, and Kilchenmann (1994), Vit, Oddo, Marano, and Mejias (1998) and Vit (2013) analyzed physicochemical composition and sensory testing of *Melipona favosa* honey to develop a common database of stingless bee honey for Venezuela. In Brazil Almeida-Muradian (2013) studied *Tetragonisca angustula* for the physicochemical properties of this honey compared with *A. mellifera* honey. In Columbia seven genera of stingless bee honeys have been analyzed (Zuluaga-Dominguez, Diaz-Moreno, Fuenmayor, & Quicazan, 2013). Ferrufino and Vit (2013) studied the chemical composition and conducted microbiological analysis on six species of stingless bee honey from Bolivia. Sgariglia, Vattuone, Sampietro Vattuone, Soberon, and Sampietro (2010) evaluated physicochemical properties and antibacterial activities of *Tetragonisca angustula* and *Plebeia wittmanni* honey from Argentina. A comprehensive review of the physicochemical properties of neo-tropical stingless bee honeys is summarized in Appendix 1 (Vit, Pedro, & Roubik, 2013). This work reviews honey analyzed from *ca.* 32 species of Central and South American stingless bee honey.

There is a paucity of research on honey chemistry reported for Indo-Australian stingless bee species and what little published research that does exist has primarily focused on the species most commonly encountered in the commercial stingless bee beekeeping industry. In Australia *Tetragonula carbonaria* honey was analyzed and compared with reported data for *A. mellifera* honey (Oddo et al., 2008). They found that relative to *A. mellifera*, the moisture content was much higher; the electrical conductivity was higher; enzyme activity was low and free acidity was much higher. The sugar composition was unusual in that the results demonstrated a low glucose and fructose content but approximately 20% maltose. For Thailand some research work has been done concerning Thai stingless bee honey. Sawattham, Vaithanomsat, and Tadakittisarn (2009) studied the composition of *A. mellifera* honey and compared this with three species of stingless bee honey (*T. pagdeni, T. laeviceps* and *L. terminata*). Their results revealed that the moisture content of stingless bee honey varied from 22.0% to 25.6% which were higher than those found in *A. mellifera* honey. Suntiparapop, Prapaipong, and Chantawannakul (2012) studied the chemical and biological properties of *T. laeviceps* honey and as others, found that when compared to *A. mellifera* honey *T. laeviceps* honey has a higher moisture content, higher acidity and lower pH. Summarizing past research findings, stingless bee honey, when compared to *A. mellifera* honey, has been shown to be higher in moisture content, lower in diastase activity, higher in electrical conductivity and acidity. The carbohydrate profile, while generally similar to *A. mellifera* can often be lower in reducing sugars. Organoleptically stingless bee honeys possess a sour–sweet (acidic) taste.

The principle objective for this study is an examination of the physicochemical properties of stingless bee honey from a cohort of species previously not examined from a geographical area (SE Asia) where past research efforts have been minimal. Here we report the results from a study of the physicochemical properties of eleven species of stingless bees indigenous to Thailand, of which several are commonly used in commercial stingless beekeeping and the remainder are species, primarily for reasons of preferred nesting habitat, unlikely to be 'managed'. Honey from the *Tetragonula* species complex taxonomically represents the majority of stingless bee honey in the commercial market in Thailand. Honey from such species as *Tetrigona melanoleuca*, *Tetrigona apicalis, Homotrigona fimbriata*, *Lisotrigona furva* and *Lepidotrigona flavibasis* would rarely enter the commercial stream of stingless bee honey. This is due largely to the nature of their natural nesting choices in cavities of mature trees (Rajitparinya, Titayavan, & Burgett, 2000). Taxonomic assignment of our stingless bee species follows Rasmussen (2008).

2. Materials and methods

2.1. Samples

Stingless bee honey samples were obtained from sealed honey pots from both natural and managed colonies during 2012, 2013 and 2014. Samples were taken in two geographical locations in Thailand; Chiang Mai province in north and from the two contiguous provinces of Chanthaburi and Trat in east central Thailand. At the time of honey sample collections, samples of adult worker bees were also taken for later taxonomic identification. The species of stingless bees from which honey was collected for analysis and their geographical origins are listed in Table 1. Honey pots were pierced with a sharp tool and honey was gravity strained through fine cloth (gauze) or syringe extraction from individual and

Table 1
Species of stingless bees from which honey was examined.

Species	Geographical origins (Thai province)	Number of samples	Type of colony
Homotrigona fimbriata	Chiang Mai	1	Natural
Lepidotrigona terminata	Chanthaburi	1	Natural
Lepidotrigona flavibasis	Chiang Mai, Chanthaburi	4	Natural and managed
Lepidotrigona doipaensis	Chiang Mai	2	Natural
Lisotrigona furva	Chanthaburi	2	Natural
Tetragonilla collina	Chiang Mai	1	Natural
Tetragonula fuscobalteata	Chanthaburi	2	Managed
Tetragonula laeviceps-pagdeni complex	Chiang Mai, Chanthaburi, Trat	10	Natural and managed
Tetragonula testaceitarsis	Chanthaburi	2	Natural and managed
Tetrigona apicalis	Chiang Mai	2	Natural
Tetrigona melanoleuca	Chiang Mai	1	Natural

collective honey pots. Honey samples were stored at 4 °C in sealed glass jars in the dark. Samples were obtained from eleven stingless bee species.

2.2. Reagents

All of the chemicals and reagents used were of analytical grade. Methanol, acetonitrile, NaOH, acetate buffer and NaCl were obtained from Sigma–Aldrich (Thailand) Co Ltd. Standards of fructose, glucose, maltose and sucrose were purchased from Sigma–Aldrich (Thailand) Co Ltd.

2.3. Analytical methods to determine physicochemical parameters in honey

Physicochemical parameters of stingless bee honey were evaluated following the AOAC official methods of analysis (2006) and International Honey Commission (2009). The parameters examined include moisture (g/100 g), ash (g/100 g), electrical conductivity (ms/cm), pH, total acidity (meq/kg), diastase activity (Gothe scale), reducing sugars (g/100 g) and hydroxymethylfurfural (mg/kg). All analyses were performed in duplicate.

2.3.1. Color

Honey color was determined by using a Hanna Honey Color HI 96785 colorimeter (Woonsocket, Rhode Island, USA). This equipment was calibrated with glycerin and color readings are expressed using the Pfund scale.

2.3.2. 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.

2.3.3. Ash content

Honey ash content was measured according to AOAC method 920.181, 2006 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 a 500 °C Muffle furnace for 2 h and then reweighed. Ash percentage was calculated.

2.3.4. Electrical conductivity

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

2.3.5. 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.

2.3.6. Diastase activity

Honey diastase activity was determined according to AOAC method 958.09, 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 spectrophotometer (Shimadzu UV-1601 UV/VIS, Japan), 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 reached.

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 h at 40 °C. The results are expressed in Gothe degrees. The use of diastase as a honey quality indicator has been questioned (White, 1994).

2.3.7. Hydroxymethylfurfural (HMF)

HMF contents in honey were determined according to AOAC method 980.23, 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 mum 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 aqueous C18 column (5 mum, 250 × 4.6 mm) (Restex, Bellefonte, Pennsylvania, USA), using as mobile phase methanol–water (10:90, v/v) at a flow rate of 1.2 mL/min. The injection volume was 20 mul, the column temperature 25 °C and the detection at 280 nm. (Mendes, Proença, Ferreira, & Ferreira, 1998).

2.3.8. Sugars

Honey sugar contents (fructose, glucose, maltose and sucrose) were determined according to AOAC method 977.20, 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 mum 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 mum, 250 × 4.6 mm) (GL Science Inc., Japan), mobile phase with HPLC acetonitrile/water (72:25) was used at a flow rate 1 ml/min, with an oven temperature of 40 °C. (Mendes et al., 1998).

3. Results and discussion

The physicochemical parameters of the honeys produced by the 11 stingless bee species studied are presented in Table 2

3.1. Color

Data from honey color analyses are expressed by Pfund units. This system of color analysis was devised by the U.S. Department of Agriculture in 1925 (Sechrist, 1925) and uses a standardized color comparator based on a scale of 1–140 mm. The Pfund scale was originally devised to develop a standardized system to classify *A. mellifera* honey color, which is an important component in determining honey quality in the commercial honey market (USDA, 1945). The data from our analysis show an average Pfund reading of 67 ± 19 mm. The honey color range for the 28 stingless bee honeys is 30–104 mm. Past research reports concerned with stingless bee honey often neglect any measure of honey color. In looking at a comprehensive analysis of U.S. honey White, Riethof, Subers, and Kushnir (1962) reported the average *A. mellifera* honey color

Table 2
Physicochemical parameters and sugar contents of stingless bee honeys from SE Asia.

Stingless bee species	Parameters												
	n	Moisture (g/100 g)	Ash (g/100 g)	Electrical conductivity (ms/cm)	pH	Total acidity (meq/kg)	Diastase activity (°Gothe)	HMF (mg/kg)	Total sugar (g/100 g)	Fructose (g/100 g)	Glucose (g/100 g)	Maltose (g/100 g)	Sucrose (g/100 g)
H. fimbriata	1	41	1.0	2.6	3.3	528	ND	46	22	7.4	15	ND	ND
L. terminata	1	30	0.245	0.780	3.5	194	0.29	ND	66	8.1	4.9	53	ND
L. flavibasis	4	28 ± 3.08	0.510 ± 0.159	1.3 ± 0.474	3.7 ± 0.098	168 ± 47	3.1 ± 1.0	8.5 ± 3.5	68 ± 3.2	16 ± 8.8	13 ± 8.6	39 ± 15	ND
L. doipaensis	2	32, 31	0.660, 0.365	1.5, 0.885	3.6, 3.4	207, 188	1.7, 1.5	2.3, ND	29, 19	15, 9	14, 9.8	ND	ND
L. furva	2	29, 27	0.325, 0.040	0.325, 0.365	3.7, 3.5	48, 58	ND	ND, 0.215	59, 66	34, 33	25, 28	ND	ND, 6.0
T. collina	1	28	0.245	0.435	3.9	25	0.340	5.9	52	26	26	ND	ND
T. fuscobalteata	2	25, 27	0.475, 0.870	1.3, 1.4	3.7, 3.7	69, 124	ND, 4.7	22, ND	42, 23	23, 19	19, 4.4	ND	ND
T. laeviceps-pagdeni complex	10	28 ± 1.8	0.220 ± 0.083	0.587 ± 0.160	3.6 ± 0.195	76 ± 30	0.630 ± 0.354	5.4 ± 8.4	66 ± 3.8	17 ± 6.7	12 ± 7.7	37 ± 12	ND, 0.025 ± 0.019
T. testaceitarsis	2	32, 29	0.270, 0.140	0.665, 0.515	3.5, 3.7	87, 54	ND, 0.220	2.4, 3.5	26, 56	15, 29	11, 27	ND	ND
T. apicalis	2	47, 37	1.0, 1.8	2.1, 3.1	3.1, 3.3	550, 440	ND, 4.9	0.260, 0.255	14, 11	6.8, 6.7	7.7, 4.1	ND	ND
T. melanoleuca	1	43	3.1	2.8	3.4	592	0.150	28	15	6.0	8.9	ND	ND
Average ± SD		31 ± 5.4	0.531 ± 0.632	1.1 ± 0.780	3.6 ± 0.198	164 ± 162	1.5 ± 1.6	8.7 ± 12	51 ± 21	17 ± 9.7	14 ± 8.6	41 ± 15	1.2 ± 2.7

ND = not detected.

to fall within a Pfund range of 27–34 mm, which is derived from sampling 504 putative mono-floral honeys. The average honey color from our analysis would grade in the color range of light amber, which falls in the Pfund range of 50–70 mm.

3.2. Moisture

Stingless bee honey is often characterized as possessing higher moisture than the paradigm of honey from the western honey bee *A. mellifera*. From the 28 samples the average moisture content is 31 ± 5.4 g/100 g. Moisture content ranged from 25 to 47 g/100 g. For the ten samples measured from the *T. laeviceps-pagdeni* complex the moisture content averaged 28 ± 1.8 g/100 g. Comparable data from South America (Vit et al., 2013) showed mean moisture content of 26 ± 4.8 g/100 g interpolated from the analysis of 31 species.

3.3. Ash

Our results provide a mean ash content of 0.531 ± 0.632 g/100 g with a range of 0.040–3.1 g/100 g. For our results an apparent outlier is the single sample of honey from *T. melanoleuca*, with an ash content of 3.1 g/100 g. All other honeys examined resulted in ash contents of <1.8 g/100 g honey. Regarding the 10 honey samples we examined from the *T. laeviceps-pagdeni* complex, an average ash content of 0.220 ± 0.083 g/100 g was derived. This is similar to the report of Suntiparapop et al. (2012) which gives an ash content for *T. laeviceps* from two adjacent Thai provinces, as 0.33 g/100 g (Chanthaburi province) and 0.20 g/100 g (Trat province). From Appendix 1 in Vit et al. (2013), we interpolated an ash content of 0.203 ± 0.265 g/100 g with a range of 0.01–1.25 g/100 g. Comparing this parameter with the results of the study by White et al. (1962) on the physicochemistry of *A. mellifera* honey, they reported an average ash content of 0.17 g/100 g from a data set of 490 samples of American honey. In a study of *A. mellifera* honey produced in northern Thailand from the most important botanical source for commercial honey in the region (Longan, *Dimocarpus longan*), an average ash content of 0.23 g/100 g was reported (Wanjai, Sringarm, Santasup, Pak-Uthai, & Chantawannakul, 2012).

3.4. Electrical conductivity

From our 28 samples the average electrical conductivity is 1.1 ± 0.780 ms/cm. Honeys from three of the 11 stingless bee species possessed conductivity reading of >2 (*T. apicalis*, 3.1 and 2.1 ms/cm; *T. melanoleuca*, 2.8 ms/cm; *H. fimbriata*, 2.6 ms/cm). Excluding these 4 honey samples from these species, the average conductivity is 0.794 ± 0.413 ms/cm. This is comparable to the report of Suntiparapop et al. (2012) who reported electrical conductivity of *T. laeviceps* from Chanthaburi and Trat provinces as 0.71 and 0.53 ms/cm. In the stingless bee honey literature originating from South America only one study (Guerrini et al., 2009) provided any information regarding electrical conductivity. This research reported an electrical conductivity of 0.48 ± 0.06 ms/cm calculated from three honey samples whose species origins were not given. Wanjai et al. (2012) reported electrical conductivity for *A. mellifera* from Thailand to be 0.26 ± 0.04 ms/cm, which is lower than our electrical conductivity for stingless bee honey.

3.5. pH

The most consistent parameter, i.e., displaying the least variability, measured in this study is the pH. From 28 honey samples examined from 11 stingless bee species, the average pH is 3.6 ± 0.198, with a range of 3.1–3.9. Ten honey samples were examined from the most commonly encountered stingless bee species (*Tetragonula laeviceps-pagdeni* complex) in Thailand and the species most frequently 'managed' by meliponiculturists. From the ten samples of this specie's honey an average pH of 3.6 ± 0.195 is revealed. In comparison to neo-tropical stingless bee species, from Vit et al. (2013), an average pH of 3.9 ± 0.601 is interpolated from 18 honey samples. The honey pH data from Thai stingless bee species reported here is slightly more acidic than that reported from South America.

3.6. Total acidity

From the 28 honey samples the average total acidity is 164 ± 162 meq/kg. The range is 25–592 meq/kg. Four honey samples from three species (*Homotrigona fimbriata*, *Tetrigona apicalis* and *Tetrigona melanoleuca*) exhibited extraordinarily high total acidity (range 440–592 meq/kg). By excluding these four honey samples, the average acidity for the 8 species remaining is 103 ± 59 meq/kg. The intra-specific total acidity (*Tetragonula laeviceps-pagdeni* complex, $n = 10$) is 76 ± 30 meq/kg with a range from 44 to 121. Our findings for this species complex are very similar to those reported by Suntiparapop et al. (2012) for samples of *T. laeviceps* from the two Thai provinces of Chanthaburi and Trat where the majority of commercial stingless beekeeping is found (Chuttong et al., 2014.)

3.7. Diastase activity

Our analytical results give an average 1.5 ± 1.6 °Gothe for the 22 honey samples where diastase was detected; six honey samples resulted in no detection of the enzyme. The range for the 22 sampled honeys is 0.050–4.9 °Gothe. Reports of diastase in South American stingless bee honeys (Vit et al., 2013) give a wide variation in diastase activity, with a range of 2.4–21 °Gothe from 14 stingless bee species. For *A. mellifera* honey from the U.S. White et al. (1962) provided diastase levels ranging from 3.1 to 22 °Gothe with an average from 15 honey samples of 17 ± 4.8 °Gothe.

3.8. Hydroxymethylfurfural (HMF)

The stingless bee honey samples showed a high level of variability both between and among species. Additionally, for a single honey sample analyzed from *Homotrigona fimbriata*, an HMF of 46 mg/kg was shown. An average HMF from all samples where HMF was detected is 8.7 ± 12 mg/kg with a range from not detected to 46. From our ten samples examined from the *T. laeviceps-pagdeni* complex, the average HMF was 5.4 ± 8.4 mg/kg, with a range of not detected to 22 mg/kg. This compares to a report from Suntiparapop et al. (2012) showing HMF averaging 0.25 ± 0.04 mg/kg and 1.9 ± 0.22 mg/kg from *T. laeviceps* honey samples of Chanthaburi and Trat (Thai provinces).

Reports concerning South American stingless bee honey (Vit et al., 2013; Appendix 1) also give HMF levels which are highly variable, from not detected to 25 mg/kg. In a study of honey from *Melipona favosa*, where 21 samples were examined, the average HMF level was reported as 18 ± 8.5 mg/kg (Vit, 2013). Historically there have been multitudinous studies concerning HMF levels in *A. mellifera* honey. One of the most comprehensive is that of White (1994) where an average HMF level from 481 *A. mellifera* honeys, was reported to be 6.2 ± 9.9 mg/kg, with a range of 0–136 mg/kg. From Thai *A. mellifera* honey, an average HMF level of 0.58 mg/kg has been reported (Wanjai et al., 2012).

HMF levels in *A. mellifera* honey have traditionally been used as a measure of honey heating history and therefore honey quality. HMF levels are also incorporated into national and international honey standards, e.g., European regional standards which require

Table 3
Average sugar composition for 11 stingless bee species honey compared to *A. mellifera* honey from the USA (White et al., 1962) and Thailand (Wanjai et al., 2012) (average sugar content in g/100 g honey).

	11 Stingless bee species honey (*n* = 28)	USA *A. mellifera* honey (*n* = 490)	Thai *A. mellifera* honey (*n* = 28)
Fructose	16 ± 8.5	38	41 ± 3.5
Glucose	13 ± 8.1	31	34 ± 3.4
Maltose	38 ± 12	7.3	Not reported
Sucrose	1.2 ± 2.6	1.3	1.9 ± 1.9
Total sugars	51 ± 21[a]	78	77

[a] Due to the absence of maltose from 8 species, and sucrose from 9 species, the combined sugar total cannot be derived from a straight forward addition of the individual sugar averages.

an average honey to attain the two limits, namely 15 and 40 mg/kg (White, 1994). Codex standard for honey (1987) requires the HMF content of honey not be more than 40 mg/kg and honey from tropical ambient temperatures shall not be more than 80 mg/kg.

3.9. Sugar

Our findings regarding carbohydrate composition (g/100 g) exhibits a great deal of variation especially between stingless bee species and from past published research this is not unexpected. And while the carbohydrate profiles are variable, it is useful to summarize our findings. Table 3 details the sugar composition of the 11 stingless bee species from 28 stingless bee honey samples we examined. The average total carbohydrate composition was 52 ± 21 g/100 g with a range of 11–72 g/100 g. From the 10 honey samples of the *T. laeviceps-pagdeni* complex, an average total sugar was 66 ± 3.8 g/100 g. Comparable data from 4 species of stingless bee honey from Guatemala (Dardon, Maldonado-Aguilera, & Enriquez, 2013) and 3 species of stingless bee honey from Columbia (Zuluaga-Dominguez et al., 2013) showed a mean total sugar content of 69 ± 8.3 g/100 g and 75 ± 1.3 g/100 g respectively. Table 4 shows the sugar content of 10 honey samples of *T. laeviceps-pagdeni* complex. The dominant sugar is maltose with an average content of 37 ± 12 g/100 g and a range from 15 to 57 g/100 g. The fructose and glucose contents were 17 ± 9.7 and 14 ± 8.6 g/100 g respectively. This is in rather sharp contrast to the report of Suntiparapop et al. (2012) who reported *T. laeviceps* honey, from southeastern Thailand, to average 29 ± 0.28 g fructose/100 g and 21 ± 0.19 g glucose/100 g honey. Additionally this report provided an average sucrose level of 19 ± 0.12 g/100 g. This again is markedly higher than our findings on sucrose which averaged 0.025 ± 0.019 g/100 g, with sucrose being non-detectable in 6 of the 10 honeys from *T. laeviceps-pagdeni* we examined. We would add that in our study, all ten samples were taken from 10 individual *T. laeviceps-pagdeni* colonies.

The carbohydrate composition for stingless bee honey from our analyses, compared to the *A. mellifera* 'standard' (Table 3) is characterized by the lower contents of monosaccharides (glucose and fructose), and elevated maltose. Sucrose was detected in only 5 of the total 28 honey samples and from only two of the 11 stingless bee species (*L. furva* and the *T. laeviceps-pagdeni* complex.) Overall, our sampled stingless bee honey displays lower content of carbohydrate compared to the western honey bee (Table 3); whereas the sugar composition of *A. mellifera* honey from Thailand (tropical) versus the USA (temperate) are very similar.

A higher level for maltose in some neo-tropical stingless bee honey has been reported by Bogdanov, Vit, and Kilchenmann (1996) with a range of 2.5–32 g/100 g. An Australia report (Oddo et al., 2008) concerning *Tetragonula carbonaria*, gave an average maltose content of 20 ± 2.9 g/100 g. For our analyses maltose is either absent (13 samples) or present at high levels (15 samples): averaging 41 ± 15 g/100 g with a range of 15–57 g/100 g. For honey from the 11 stingless bee species, maltose was detected in only 3 species (*L. terminata, L. flavibasis, T. laeviceps-pagdeni* complex), but when present it was the dominant sugar. While maltose is not unknown in *A. mellifera* honey (White et al., 1962) and has been reported previously in stingless bee honey from South America (Bogdanov et al., 1996; Oddo et al., 2008) when present it is normally in small amounts.

Table 4
T. laeviceps-pagdeni honey sugar content (g/100 g).

Sample number	Sugars content (g/100 g)				
	Fructose	Glucose	Maltose	Sucrose	Total sugars
1	23	20	21	ND	65
2	12	8.8	42	0.012	63
3	29	26	15	0.050	71
4	24	20	27	0.033	71
5	12	8.3	42	0.013	63
6	15	10	37	ND	63
7	10	5.4	46	ND	62
8	14	10	39	ND	64
9	8.6	3.2	57	ND	69
10	13	9.2	38	ND	61
Mean ± SD	16 ± 6.8	12 ± 7.3	36 ± 12	0.027 ± 0.018	65 ± 3.7

ND = not detected.

4. Conclusions

The two primary goals of these investigations were to compare honeys from an expanded number of stingless bee species known in Thailand (11 of the described 32 species) and to examine intra-specific variability in the one species most frequently used in commercial stingless bee beekeeping (*T. laeviceps-pagdeni* complex).

A summarized physicochemical profile of the 28 honey samples, while exhibiting variability, especially between species, conforms to previous finding for stingless bee honey from South America, but with some noted variations. When compared to the cosmopolitan *A. mellifera* honey standards, Thai stingless bee honey revealed a higher moisture content (31 g/100 g); higher ash content (0.531 g/100 g); lower pH (3.6); higher acidity (164 meq/kg); lower diastase activity (1.5 °Gothe); slightly elevate HMF (6.6 mg/kg) and quantitatively lower total carbohydrates (total sugars, 51 g/100 g).

Concerning the sugar spectrum, the disaccharide maltose was detected in only 3 of the 11 stingless bee species we examined, but when present it was frequently the dominant carbohydrate.

Our in-depth look at multiple samples from *T. laeviceps-pagdeni* honey, a Southeast Asian species where previous research on its honey composition has been undertaken (Suntiparapop et al., 2012) gives similar results to past research except for the parameters of HMF (higher) and lower levels of the reducing sugars (glucose and fructose). A noticeable difference from our carbohydrate analysis of *T. laeviceps-pagdeni* species complex honey is the near absence of sucrose which is contrary to the report of Suntiparapop et al. (2012) who examining honey from the same stingless bee species from the same geographical region of Thailand where the majority of our *T. laeviceps-pagdeni* samples originated.

Our analyses of honey from *Tetrigona melanoleuca*, *Tetrigona apicalis*, *Homotrigona fimbriata*, *Lisotrigona furva*, *Tetragonula fuscobalteata*, *Tetragonula testaceitarsis*, *Tetragonilla collina*, *Lepidotrigona flavibasis* and *Lepidotrigona doipaensis* are the first known reports on the physicochemistry from these SE Asian stingless bee species.

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References

A.O.A.C. (Association of Official Analytical Chemist) (2006). In W. Horwitiz (Ed.), Official methods of analysis of the AOAC, 18th ed. Washington D.C., USA: Association of Official Analytical Chemists.

Almeida-Muradian, L. B. (2013). *Tetragonisca angustula* pot-honey compared to *Apis mellifera* honey from Brazil. In P. Vit et al. (Eds.), *Pot-honey a legacy of stingless bees* (pp. 375–382). New York: Springer.

Ayala, R., Gonzalez, V. H., & Engel, M. S. (2013). Mexican stingless bees (Hymenoptera: Apidae): diversity, distribution, and indigenous knowledge. In P. Vit et al. (Eds.), *Pot-honey a legacy of stingless bees* (pp. 135–152). New York: Springer.

Bogdanov, S., Vit, P., & Kilchenmann, V. (1996). Sugar profiles and conductivity of stingless bee honeys from Venezuela. *Apidologie, 27*, 445–450.

Chuttong, B., Chanbang, Y., & Burgett, M. (2014). Meliponiculture – stingless bee beekeeping in Thailand. *Bee World, 91*(2), 41–45.

Codex Alimentarius Commission (1987). Revised codex standard for honey codex standards. 12-1981. Rev, 1, 1–7.

Codex Alimentarius Commission (2001). Alinorm 41/10: Revised standard for honey, Alinorm 1, 19–26.

Crane, E. (1992). The past and present status of beekeeping with stingless bees. *Bee World, 73*, 29–32.

Dardon, M. J., Maldonado-Aguilera, C., & Enriquez, E. (2013). The pot-honey of Guatemala bees. In P. Vit et al. (Eds.), *Pot-honey a legacy of stingless bees* (pp. 395–408). New York: Springer.

Ferrufino, U., & Vit, P. (2013). Pot-honey of six Meliponines from Amboro National Park, Bolivia. In P. Vit et al. (Eds.), *Pot-honey a legacy of stingless bees* (pp. 409–416). New York: Springer.

Guerrini, A., Bruni, R., Maietti, S., Poli, F., Rossi, D., Paganetto, G., et al. (2009). Ecuadorian stingless bee (Meliponini) honey: A chemical and functional profile of an ancient health product. *Food Chemistry, 114*, 1413–1420.

International Honey Commission (2009). Harmonised methods of the international honey commission (63 pp). Swiss Bee Research Centre, Bern: FAM, Liebefeld.

Mendes, E., Proença, E. B., Ferreira, I. M. P. L. V. O., & Ferreira, M. A. (1998). Quality evaluation of Portuguese honey. *Carbohydrate Polymers, 37*(3), 219–223.

Michener, C. D. (2000). *The bees of the World*. Baltimore: Johns Hopkins University Press. 913 pp.

Michener, C. D. (2013). The Meliponini. In P. Vit et al. (Eds.), *Pot-honey a legacy of stingless bees* (pp. 3–17). New York: Springer.

Oddo, L. P., Heard, T. A., Rodríguez-Malaver, A., Pérez, R. A., Fernández-Muiño, M., Sancho, M. T., et al. (2008). Composition and antioxidant activity of *Trigona carbonaria* honey from Australia. *Journal of Medicinal Food, 11*(4), 789–794.

Rajitparinya, T., Titayavan, M., & Burgett, M. (2000). The ecology and diversity of stingless bees (Hymenoptera: Apidae) in northern Thailand. In *Proceedings seventh international conference on tropical bees: management and diversity* (pp. 91–95): IBRA.

Rasmussen, C. (2008). Catalog of the Indo-Malayan/Australian stingless bees (Hymenoptera: Apidae: Meliponini). (Zootaxa 1935). (80 pp). Auckland: Magnolia Press.

Rosales, G. R. O. (2013). Medicinal uses of *Melipona beecheii* honey, by the ancient Maya. In P. Vit et al. (Eds.), *Pot-honey a legacy of stingless bees* (pp. 229–240). New York: Springer.

Sawattham, A., Vaithanomsat, P., & Tadakittisarn, S. (2009). Comparative composition of honey from Thai stingless bee and European honey bee (Apis mellifera L.). In *Proceedings of 47th Kasetsart University annual conference: Plants, 17–20 March 2009* (pp. 139–144).

Sechrist, E. L. (1925). The color grading of honey. USDA Dept. Circ. 364, October 1925.

Sgariglia, M. A., Vattuone, M. A., Sampietro Vattuone, M. M., Soberon, J. R., & Sampietro, D. A. (2010). Properties of honey from *Tetragonisca angustula fiebrigi* and *Plebeia wittmanni* of Argentina. *Apidologie, 41*, 667–675.

Souza, B., Roubik, D., Barth, O., Heard, T., Enriquez, E., Carvalho, C., et al. (2006). Composition of stingless bee honey: Setting quality standards. *Interciencia, 31*, 867–875.

Suntiparapop, K., Prapaipong, P., & Chantawannakul, P. (2012). Chemical and biological properties of honey from Thai stingless bee (*Tetragonula laeviceps*). *Journal of Apicultural Research, 51*(1), 45–52.

USDA (1945). United States Standards for Grades of Extracted Honey. U.S. Department of Agriculture, Agricultural Marketing Service, issued January 30, 1943 (re-issued December 15, 1945).

Vit, P., Bogdanov, S., & Kilchenmann, V. (1994). Composition of Venezuelan honeys from stingless bees and *Apis mellifera* L. *Apidologie, 25*, 278–288.

Vit, P., Oddo, L. P., Marano, M. L., & Mejias, E. S. (1998). Venezuelan stingless bee honey characterized by multivariate analysis of physicochemical properties. *Apidologie, 29*, 377–389.

Vit, P., Pedro, S. R. M., & Roubik, D. W. (Eds.). (2013). *Pot-honey a legacy of stingless bees*. New York: Springer (Appendix I).

Vit, P. (2013). *Melipona favosa* pot-honey from Venezuela. In P. Vit et al. (Eds.), *Pot-honey a legacy of stingless bees* (pp. 363–373). New York: Springer.

Wanjai, C., Sringarm, K., Santasup, C., Pak-Uthai, S., & Chantawannakul, P. (2012). Physicochemical and microbiological properties of longan, bitter bush, sunflower and litchi honeys produced by *Apis mellifera* in northern Thailand. *Journal of Apicultural Research, 51*(1), 36–44.

White, J. W. (1994). The role of HMF and diastase assays in honey quality evaluation. *Bee World, 75*(3), 104–117.

White, J. W., Riethof, M. L., Subers, M. H., & Kushnir, I. (1962). Composition of American honeys (No. 1261). US Department of Agriculture.

Zuluaga-Dominguez, C. M., Diaz-Moreno, A. C., Fuenmayor, C. A., & Quicazan, M. C. (2013). An electric nose and physicochemical analysis to differentiate Columbian stingless bee pot-honey. In P. Vit et al. (Eds.), *Pot-honey a legacy of stingless bees* (pp. 417–428). New York: Springer.