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A simple approach for rapid detection and quantification of adulterants in stingless bees (*Heterotrigona itama*) honey

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ABSTRACT

In this study, we propose an easy approach by combining the Fourier transform infrared and attenuated total reflectance (FTIR-ATR) spectroscopy together with chemometrics analysis for rapid detection and accurate quantification of five adulterants such as fructose, glucose, sucrose, corn syrup and cane sugar in stingless bees (*Heterotrigona itama*) honey harvested in Malaysia. Adulterants were classified using principal component analysis and soft independent modeling class analogy, where the first derivative of the spectra in the wavenumber range of 1180–750 cm^{-1} was utilized. The protocol could satisfactorily discriminate the stingless bees honey samples that were adulterated with the concentrations of corn syrup above 8% (w/w) and cane sugar over 2% (w/w). Feasibility of integrating FTIR-ATR with chemometrics for precise quantification of the five adulterants was affirmed using partial least square regression (PLSR) analysis. The study found that optimal PLSR analysis achieved standard error of calibrations and standard error of predictions within an acceptable range of 0.686–1.087% and 0.581–1.489%, respectively, indicating good predictive capability. Hence, the method developed here for detecting and quantifying adulteration in *H. itama* honey samples is accurate and rapid, requiring only 7–8 min to complete as compared to 3 h for the standard method, AOAC method 998.12.

1. Introduction

Stingless honey bees belonging to the tribe of *Meliponini*. Such honey bees are distinct from the genus *Apis* in terms of morphology (absence of sting), nectar collection, short harvest distance in searching food and their honeycomb-less hives (Vit, Pedro, & Roubik, 2013). Apart from their robustness, rearing stingless bees are more preferable to the Malaysian bees' farmers because their population is less susceptible to harsh environments and seasonal changes (Kelly, Farisya, Kumara, & Marcela, 2014). There are two species of stingless bees being commonly domesticated in Malaysia, namely the *Heterotrigona itama* and *Geniotrigona thoracica* (Razak, Aziz, Ali, Ali, & Visser, 2016). Current estimate revealed that 90% of honey from stingless bees sold in Malaysia is obtained from *Heterotrigona itama* (Kelly et al., 2014). The market price of such honey can be as high as $100/kg (Shadan, Mahat, Wan Ibrahim, Ariffin, & Ismail, 2017), which is nearly 2 times higher than the one produced by *Apis mellifera* ($20–40/kg). Stingless bee honey is expensive because of the exceptionally higher contents of flavonoids and polyphenols as compared to honey produced by the *Apis* spp. (Biluca, Braghini, Gonzaga, Costa, & Fett, 2016; Rodriguez-Malaver, 2013; Rodriguez-Malaver et al., 2009). Most of the beneficial characteristics of flavonoids and polyphenols found in stingless bee honey can be ascribed to their excellent antioxidant properties which is a fundamental aspect important to life (Silva et al., 2013). Stingless bees honey is popularly known for its therapeutic applications to cure or manage various diseases/complications including throat inflammation, gastritis, cataract, post-birth recovery, *etc.* (Vit, Medina, & Enriquez, 2004). For such reasons, researchers extensively studied the physicochemical properties and biological activities of stingless bees honey (Sousa et al., 2016; Biluca et al., 2016; Chuttong, Chanbang, Sringarm, & Burgett, 2016). Sousa et al. (2016) found the water content in stingless bees honey was higher than those found in honey of sting bees (*Apis mellifera*). Interestingly, Biluca et al. (2016) reported that the sugar composition of ten samples of Brazilian stingless bees honey were similar to those of *Apis mellifera* honey, whereas another study by Chuttong et al. (2016) indicated, otherwise. These contradictory

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findings suggest that the sugar profile of honey from stingless bees can vary from one region to another, depending on the flora and vegetation that predominates the region.

Considering the limited production, lacking of international standard as well as the high economic and therapeutic value of stingless bees' honey, certain profiteering individuals have come to realized that their profits can be maximized by adulterating pure honey with cheap and easily available sweeteners and selling them at premium prices. Such acts are widespread worldwide and various reports revealed cheaper sweeteners including high fructose corn syrup, glucose syrup, corn syrup, inverted syrup and cane sugar are commonly used as additives in honey (Wu et al., 2017), where the percentages of adulteration are varied from 2to 27% (Sivakesava & Irudayaraj, 2001). In order to check the purity of the honey, numerous analytical techniques have been developed to detect and quantify the adulterants present in the honey that are sold on various premises. These techniques include nuclear magnetic resonance (NMR) spectroscopy (Ribeiro et al., 2014), high-performance liquid chromatography (HPLC) (Wang et al., 2015), gas chromatography (GC) (Ruiz-Matute, Soria, Martinez-Castro, & Sanz, 2007) and stable carbon isotope ratio analysis (Tosun, 2013). However, for high-throughput routine analysis of honey adulterations these techniques are not suitable because of their destructive nature, requirement of long analysis time and expensive reagents. To overcome these shortcomings, development of a simple, fast, economic and yet accurate alternative technique is necessary. Such user-friendly method will enable swift monitoring of the purity of stingless bees' honey sold in the open market. In the past, Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectroscopic technique has been widely applied for molecular fingerprinting (Smith, 2011). Meanwhile, it is realized that the performance of FTIR-ATR spectroscopy can further be enhanced by combining with chemometrics.

Driven by this idea, we proposed a simple method by integrating chemometrics with FTIR-ATR spectroscopy for quick detection and quantification of incorporated adulterants in stingless bee (*H. itama*) honey. Being an inexpensive and non-invasive procedure, the developed method permits a rapid examination of honey without requiring any laborious process for sample preparation. In this study, the FTIR-ATR spectra were obtained for both pure and adulterated *H. itama* honey. Standard sugars (fructose, glucose and sucrose) and commercial sugars (corn syrup and cane sugar) were used as adulterants. Based on the obtained FTIR-ATR spectral data, a chemometric model was built for further data analysis. Previously, the integration of FTIR-ATR with chemometrics data analysis was assessed by several researchers for the quantification of sugar profile (Anjos, Campos, Ruiz, & Antunes, 2015), determination of geographical origin (Gok, Severcan, Goormaghtigh, Kandemir, & Severcan, 2015) and evaluation of adulterants present in conventional sting bees honey (Rios-Corripio, Rojas-Lopez, & Delgado-Macuil, 2012; Wang, Kliks, Jun, Jackson, & Li, 2010; Gallardo-Velazquez, Osorio-Revilla, de Loa, & Rivera-Espinoza, 2009). Gallardo-Velazquez et al. (2009) and Wang et al. (2010) noticed differences in the FTIR-ATR spectra of adulterated honey and they further predicted the extent of adulteration using partial least square regression (PLSR) analysis. Rios-Corripio et al. (2012) introduced the use of principal component analysis (PCA) on the obtained FTIR-ATR spectra which showed clear discrimination between the pure and adulterated honey. While several works have used FTIR-ATR for authentication of honey (Rios-Corripio et al., 2012; Wang et al., 2010; Gallardo-Velazquez et al., 2009), the limit of detection (LOD) for differentiating the pure and adulterated honey were not described in these studies. Moreover, the effectiveness of this technique for simultaneous detection and quantification of adulterants in stingless bee (*H. itama*) honey has not been performed. Therefore, for the first time, this study aims to determine the capability of chemometrics integrated FTIR-ATR spectroscopy for detection and quantification of various adulterants present in stingless bee (*H. itama*) honey.

Table 1
Origin, label and year of harvest of stingless bee (*H. itama*) honey samples.

Location in Malaysia	Sample label/harvest year
Jabi, Terengganu (JBT)	JBT1/2016, JBT2/2014, JBT3/2014
Bukit Kor, Terengganu (BKT)	BKT1/2016, BKT2/2014
Paka, Terengganu (PT)	PT/2016
Jerteh, Terengganu (JTT)	JTT1/2014, JTT2/2014
Marang, Terengganu (MRT)	MRT1/2014, MRT2/2014, MRT3/2014, MRT4/2014, MRT5/2014, MRT6/2014
Kota Bahru, Kelantan (KBK)	KBK/2016
Yan, Kedah (IMK)	IMK/2016

2. Materials and methods

2.1. Sampling of honey

Present study used a total of sixteen pure *H. itama* honey samples collected from established bee farms in the Northern region of Peninsular Malaysia (fourteen from Terengganu, one from Kedah and one from Kelantan). As the farming of stingless bees in Malaysia is a growing industry and the commodity has yet to be regulated according to the international standard, all samples were self-collected from reliable local bee farms that were also community partners of the School of Food Science and Technology, Universiti Malaysia Terengganu. To ensure purity of the honey samples, the collection was performed on colonies that were free from any exogenous sugar feeding throughout the apiary distance of 100 m. Table 1 enlists the studied stingless bees honey samples origin, label and year of harvest. Five honey samples were collected in October 2016 and eleven samples were obtained from different bees' hives during the month of July in 2014. All batches of honey were stored under room temperature (26 ± 2 °C) prior to the adulteration and subsequent analytical measurements.

2.2. Preparation of sugar standards and adulteration of honey

Two types of sweeteners were used as adulterants of the *H. itama* honey such as standard sugar and commercial sugar. Analytical grade standard sugar adulterants including D(−)-fructose, D(+)-glucose and D(+)-sucrose and sugars, D(−)-fructose were purchased from Systerm, China while D(+)-glucose and D(+)-sucrose were purchased from QRec, Malaysia. Commercial sugars such as corn syrup and granulated cane sugar were purchased from local confectionary shops and stores. Corn syrup and granulated cane sugar were selected as possible adulterants due to their low prices and easy availability. Other types of sugar adulterants were not considered in this study as they were expensive and not readily available in Malaysia. The sugar solutions were prepared by diluting an appropriate amount of sugar with ultrapure water (Milli Q system, Millipore) and adjusted to 70 °Brix.

To achieve good statistical accuracy, three samples of pure *H. itama* honey from different states of Malaysia (Kedah, Kelantan, and Terengganu, hereafter labeled as IMK, KBK and PT, respectively) were randomly selected for adulteration, while other samples were kept in the pure form. From the three pure honey samples, a total of 180 and 120 batch samples were prepared for the adulteration with standard sugar and commercial sugar, respectively. Adulteration was performed by adding an adulterant solution (previously adjusted to 70 °Brix) into pure honey samples at 3% (w/w) incremental steps. For every adulterant the concentrations were varied from 2 to 59% (w/w) to get total 60 samples in each batch. All adulterated samples were stirred at 300 rpm for 5 min using a magnetic stirrer to achieve excellent homogeneity of the solution.

2.3. FTIR-ATR measurement

Mid-infrared (MIR) spectra in the wavenumber range of 4000 to