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INTRODUCTION

Food authentication is importance for both consumers and industries, involve to food processor that do not desire to be subjected to unfair competition from dishonest processors. Moreover, authentication of food is vital for consumer who would obtain an economic advantage from the misrepresentation of the food they are selling (Cordella et al., 2002; Gallardo-Velázquez et al., 2009). Honey bee products is defined as the natural and nutraceutical product, proposes as healthy food and rich of nutrition because honey compose of various chemical components (Wang et al., 2010). Nowadays, honey bee could be an important income source for the beekeeper, as well as the renting of colonies for pollination service (Sawatthum, 2004). Therefore, there is an international effort to standardize the quality parameters of these honeys.

Stingless bees, the subfamily Meliponinae, are an important role as insect pollinator of flowering plants in the tropics (Truchado et al., 2011, Sawatthum et al., 2009). These stingless bee honeys are interesting due to their attracting as health-promoting food products and in pharmacy, although their constituents have little been studied in detail due to the scarcity of samples and the complexity of their composition (Truchado et al., 2011). The chemical constituents of most honey consists of water, glucose, fructose, maltose, sucrose, and other minor compounds involving proteins, organic acids, vitamins, flavonoids and acetylcholine (Wang et al., 2010). The carbohydrates are the major components of honey, including monosaccharides fructose and glucose which account for 85-95% of the honeybee honey sugars (Bogdanov et al., 1996). The sugar profile is those quality criteria, which are best suitable for differentiating different sorts of honey. However, there are several analytical methods used to apply the chemical, physical and biomolecular constituents in honey, the methods are expensive, produced under wide fluctuations in weather and harvesting conditions are particularly susceptible (Ruoff et al., 2006a; Ruoff et al., 2006b).

Fourier-transform infrared (FTIR) microscopy technique is a vibrational technique, giving the increased performance of computers of infrared spectrometry that has become a rapid and well-established technique for quantitative food analysis (Gao et al., 2000; Ruoff et al., 2006a). The use of FTIR spectroscopy and multivariate analysis has advantages over other techniques and provides a rapid, robust, inexpensive qualitative analytical method to assess the qualities of various food samples. Previous studied of Wang et al. (2010) found, FTIR spectroscopy enabled qualitative and qualitative analyses of glucose, fructose, sucrose, and maltose in multiple regional honey samples.

This research aims to discriminate the major composition of royal honey bee from 3 Thai stingless honey bee species. Furthermore to apply FTIR-microscopy technique as novel tool to predict concentrations of sugars in Thai stingless bee and honey samples from commercial markets and the model predictions will be verified by comparing with HPLC technique.

MATERIALS AND METHODS

Honey samples

Honey samples of four species [*Apis dorsata* Fabricius; Royal honey, and Three Thai stingless honey bee; *Trigona pagdenis* Schwarz (Khon-Ngen), *Trigona laeviceps* Smith (Pak-Trae) and *Trigona* sp. (Pak-Trae-White)] were collected from beekeepers from Chanthaburi province, Thailand. All honey samples were stored at 4 °C before analysis.

FTIR microspectroscopy

The infrared spectra of 4 honey samples were fixed on low-e slides (MirrIR, Kevley Technologies). After that, the fixed honey samples were dried and kept in desiccators until determined by FTIR microscopy technique. Measurements were performed at the IR end station, Synchrotron Light Research Institute (Public Organization), Thailand. The Bruker Hyperion 2000 microscope (Bruker Optics Inc., Ettlingen, Germany) equipped with a nitrogen cooled MCT (HgCdTe) detector with a 36×IR objective, coupled to the Bruker Vertex 70 spectrometer was used for IR data acquisition. All the spectra were acquired at 4.0 cm^{-1} resolution with 32 scans co-added in a spectra range from 4000 to 600 cm^{-1} (Bruker Optics Ltd, Ettlingen, Germany). The

spectrums were analyzed by using OPUS 6.5 (Bruker Optics Ltd, Ettlingen, Germany) and Unscambler program (Heraud et al., 20005).

An FTIR microscope simultaneously acquired over 100 spectra from the honey samples. Selected IR spectral regions were analyzed by multivariate data analysis, principal components analysis (PCA). The significant variation between the data sets was identified by PCA with the spectral range of 3000-2800 cm^{-1} and 1500-700 cm^{-1} by using Unscrambler software (version 9.7, CAMO Software AS, Oslo, Norway). Data manipulation were processed by taking the second derivative using the Savitzky–Golay algorithm with nine points of smoothing which allow to minimizing the effects of variable baselines and normalized with multiplicative signal correction (EMSC) which normalizes spectra, accounting for differences in sample thickness. Six PCs were chosen for analysis. Score plots (2D) and loading plots display the clustering and the variation of data set, respectively. The peak areas integration was conducted by OPUS 6.5 software. After that, the integrated peak area was used to process and represented by the Histogram.

RESULTS AND DISCUSSION

The FTIR spectra of royal honey and three stingless honey bee (*Trigona* spp.; *T. pagdenis, T. laeviceps* and *Trigona sp.*) with the corresponding band assignments was present in Figure 1A. The spectral bands are representative of the chemical groups of components present in the sample as corresponding to previous reports (Gallardo-Velázquez et al., 2009; Tewari and Irudayaraj, 2004). The assignment of each functional groups corresponding to the vibration modes was based on designation of the spectrum peaks and matching the frequency with the corresponding chemical group that absorbs in the IR regions. First region is the absorbance bands a broad, strong NH_3^+stretching band in the 3000 to 2700 cm^{-1} region which assigned to 2953 cm^{-1} of honey and can be related to primary amino acids (Silverstein and Webster 1998). The integral area from primary analysis spectra of amino acid (2953 cm^{-1}) region of *Trigona sp.* was higher than royal honey, T. *pagdenis* and *T. laeviceps*, repectively (Figure 1B). Second region, the spectra of 700 to 1500 cm^{-1} fingerprint region could be observed three major sugar constituents of honey such as fructose, glucose, and sucrose region. The peak around 1327 cm^{-1} may be due to O-H bending of the C-OH group, and the band at 1402 cm^{-1} may be due to a combination of O-H bending of the C-OH group and C-H bending of alkenes (Gallardo-Velázquez et al., 2009). The bands in the 1091 to 1153 cm^{-1} region are assigned to C-O and C-C stretching modes (Hineno, 1977), and those around 1474 to 1199 cm^{-1} are due to the bending modes of O-C-H, C-C-H, and C-O-H angles. The peak at 935 cm^{-1} is due to the C–H bending of the carbohydrate, whereas the peaks observed at 1093 and 1274 cm^{-1} correspond to the C–O stretch in the C–OH group as well as the C–C stretch in the carbohydrate structure, the peak at 1119 cm^{-1} has been assigned to stretching of the C–O band of the C–O–C linkage, which present in sucrose as a glycoside bond (Tewari & Irudayaraj, 2004; Sivakesava & Irudayaraj, 2001;). The saccharide configurations and the anomeric region is assigned from 900 to 750 cm^{-1} (Tul'chinsky et al. 1976; Tewari & Irudayaraj, 2004). Histogram of Figure 1B show the second region which are alkenes (1400-1337 cm^{-1}), carbohydrate (1239-1014 cm^{-1}), sugar glycoside (915-1016 cm^{-1}) bond and saccharide (915-700 cm^{-1}), data of these integral area of *Trigona sp.* was shown higher than other honey samples. The two water absorption, O-H stretching band (wave number 1547–2370cm^{-1} and 3100–3886 cm^{-1}) were not used for filter selection because of the strong noise of the signal (Chen and Irudayaraj 1998).

The principal component analysis (PCA) was used to analyze the spread of the datasets acquired from four different groups of the experiment. PCA was performed on second derivative spectra from 37, 50, 89, 81 spectra acquired from the royal honey, *T. pagdenis, T. laeviceps* and *Trigona sp.*, respectively. Figure 2A illustrated that the clusters of the royal honey bee was well separated from the clusters of three species of *Trigona spp.* along PC-1 (34%). Analysis of PCA loading plots (Fig. 2B&C) was used to determine regions of the FTIR spectrum which most contribute to the clustering observed in the score plots (Fig. 2A). Especially for the region of sugar (1400-700 cm^{-1}) was used to discriminate royal honey bee from 3 species of *Trigona spp* at 34% PC-1 loading. The spectra of *Trigona sp.* honey bee were significantly distinct from the clusters of the royal honey, *T. pagdenis* and *T. laeviceps* honey bee along PC-2 (17%) as showed in Figure 2A & C.

An important advantage of FTIR microscopy and data analysis using PCA is the ability not only to discriminate whether a honey sample does belong to species but also to determine the differentiation of chemical constituent in honey bee. Moreover, class prediction in PCA analysis could fall into the sample was properly classified into the predefined species of the honey: *Trigona sp* or Royal honey bee.

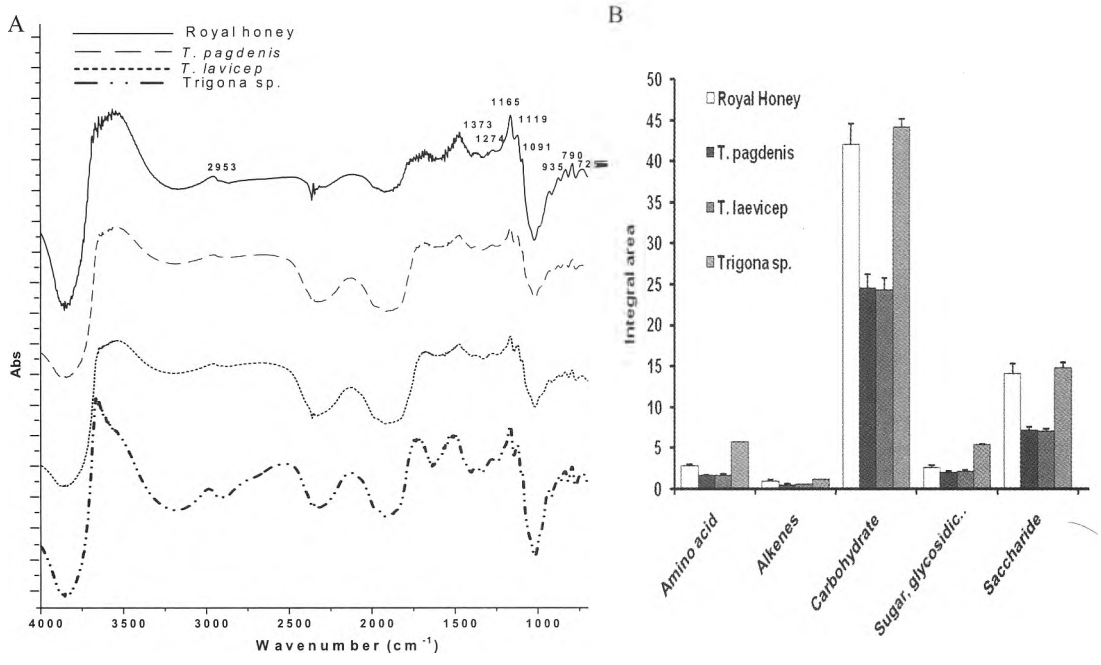

Figure 1 Analysis of four honey bee samples using FTIR microspectroscopy. (A) Average FTIR spectra obtained from different 4 honey bee: the original infrared spectral of (—) royal honey bee, (- - -) *T. pagdenis*, (----) *T. laeviceps* and (-···-) *Trigona sp.* (B) Histogram shows mean integrated areas for remarkable amino acid and other sugar regions of the original spectra. The differences between the means of areas for these regions were significantly difference. Error bars indicate standard errors of the means of triplicate average spectra.

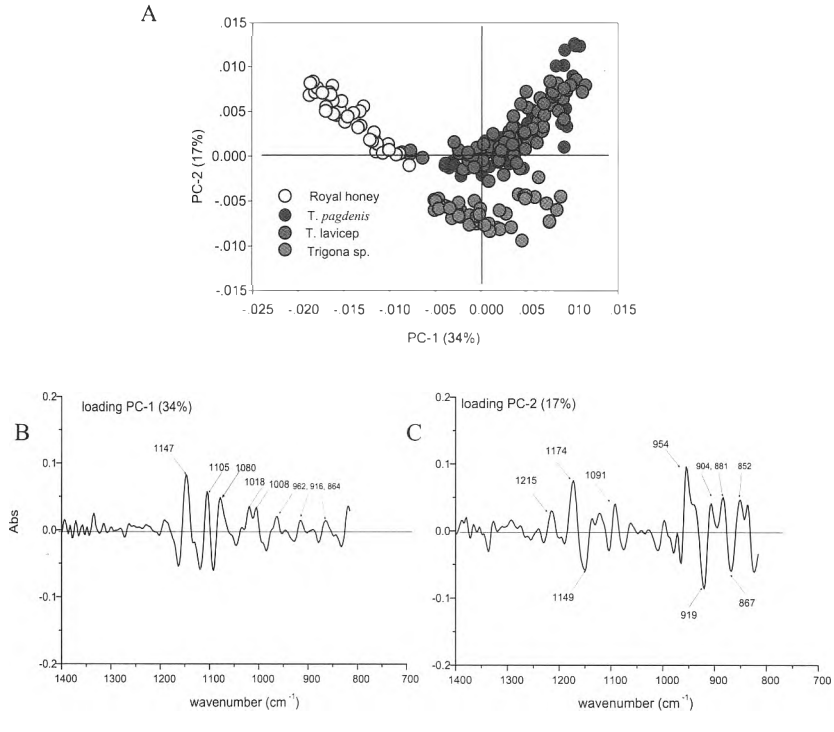

Figure. 2 PCA analysis of the FTIR spectral range 1500-900 cm^{-1} giving PCA plots (A) and PCA loading (B &C).

CONCLUSION

The preliminary study for apply the FTIR microscopy technique for discrimination the royal honey bee from other Thai stingless honey bee. The results were concluded that this technique might be a suitable method for the simultaneous and discrimination the different species of honey bee. Base on the data analysis the FTIR spectroscopy and PCA analysis is proved to be an alternative potential method to yield a signature for chemical composition of the royal honey bee with other stingless honey bee species samples. However, more detail of chemical constituent of the honey samples should be future studied. There is still lot of work to be done, to harmonize the techniques and selection of principal criteria to be used for a reproducible and reliable determination of the botanical origin of honey. FTIR seems to be rapid, non-destructive, highly sensitive and accurate quantitative analysis of honey. However, many different FTIR instruments are used for honey control with calibrations being based on different software and experimental protocols. Therefore a standardization of the method as it has been performed for further performed and compared to other standard methods.

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