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Original Article

Stevioside content in stingless bee (*Heterotrigona itama*) honey in Stevia farming

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Abstract

A study on the efficacy of stingless bee (*Heterotrigona itama*) rearing in *Stevia rebaudiana* farming was conducted at Pusat Tunas Stevia (PTS), Kampung Tempinis, Jabi, Terengganu from January until December 2015. Integration honey of stingless bee and stevia is a new approach in honey industry. Stingless bee colonies were placed in stevia farm so that, stingless bee roamed and visited for nectar collection naturally thus produced natural stevia stingless bee honey. The combination of honey and stevia which is high in nourishment is good for health with enhancement of its sweetness. The objective of this research was to produce the designer stingless bee honey that contained stevioside, a compound from stevia. Peak of stevioside was detected at retention time 1.383 via High Performance Liquid Chromatography (HPLC) analysis and peaks with identical retention time found in samples. Stingless bees also foraged nectar from herb such as stevia plant by proving the stevioside content in stingless bee honey. In conclusion, this study can help the community to improve their lifestyle and income indirectly, by increase value of honey and can export to abroad and contribute a lot of benefits to many sectors in Malaysia such as stingless bee keeper and government to increase the economy of honey sector.

Keywords: Stevia, Stingless bee, Stevioside, Kelulut, Terengganu

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Introduction

An integration of stingless bee (*Heterotrigona itama*) and *Stevia rebaudiana* is a special project which to produce a unique stingless bee honey by design the taste of honey. Stingless bee belongs to family Apidae and tribe Meliponini. Stingless bee is a eusocial insect that lives in colonies of a few hundred to thousand workers (Sakagami, 1982). Individual colony is generally perennial and has reported maximum life spans ranging from 10-26 years (Wille, 1983; Roubik, 1989). Some species of stingless bee are present in the rain forest, savanna and also transitions between forest and savanna vegetation zones (Bortoli and Laroca, 1990). *Stevia rebaudiana* is originating from the Asteraceae family, herbaceous perennial plant with extensive root system which has alternate leaf arrangement and herbaceous growth habit (Singh and Rao, 2005). Stevia plants are conventionally propagated through cutting (Khalil et al., 2014). Stevia

leaves contain diterpene glycosides, stevioside and rebaudioside (Cardello et al., 1999). Stevioside is the main sweet component, followed by other compound that present in a lower concentration such as steviolbioside, rebaudioside and dulcoside A10 (Kennelly, 2002). The main advantages of stevioside as a dietary supplement for human including its stable properties, non-calorie, maintaining good dental health, alternative sweetener for diabetic, phenylketonuria patients and obese persons (Geuns, 2003).

Material and Methods

Material and reagents

Stevioside standard (Stevioside hydrate from Stevia sp. ≥98% (HPLC) powder), acetonitrile HPLC grade, methanol, deionized water adjusted pH 2.5, deionized water, C18 SPE cartridges (Bond Elut C18, Agilent), HPLC column Zorbax SB-C18 (4.6 x 150mm, 5 µm, Agilent Technologies).

Stevioside extraction

Honey samples were collected from Pusat Tunas Stevia (PTS), Kampung Tempinis, Jabi, Terengganu, where samplings have been done once every month for a year. The SPE cartridges were conditioned 3ml of acetonitrile followed by 3ml of methanol to activate the SPE cartridge and equilibrated with 3ml of deionized water (pH 2.5) prior to sample loading. Sample loading of 1 ml diluted honey (honey: deionized water, ratio 1:1) were done and the elution of the samples were collected for drying process via nitrogen air blow. Then, the dried samples were re-dissolved in 1ml deionized water for HPLC analysis.

HPLC analysis

The separations and stevioside compound in samples were carried out on an HPLC (Shimadzu, UV detector at 210nm). The data were collected and analyzed by Shimadzu Lab Solution®. The analytical column was Zorbax SB-C18 (4.6 x 150mm, 5 µm). The mobile phase consisted of acetonitrile: water 80:20 (v/v). Gradient elution was used is 11 minutes. The system was operated at room temperature and the flow rate was 1mL/min and detected at wavelength 210 nm. Injection volume is 20µm. The samples are compared with standard stevioside (50ppm, diluted in deionized water).

Results and Discussion

The stevioside composition in honey samples from PTS Kampung Tempinis, Jabi, Terengganu were analysed and presented in chromatograph with the UV detector at wavelength 210nm. The stingless bee honey samples compared to the stevioside standard for the stevioside analysis in stingless bee honey. Figure 1 shows the peak of stevioside standard at retention time 1.38.

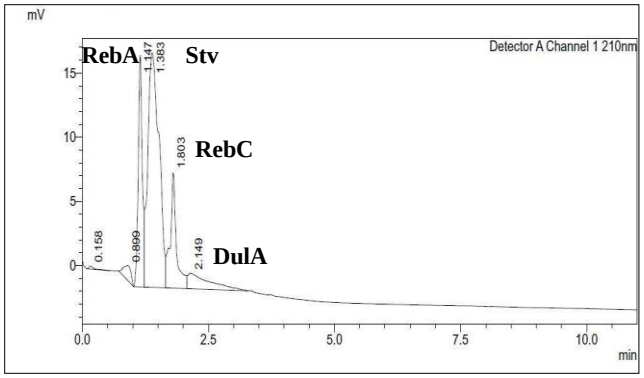
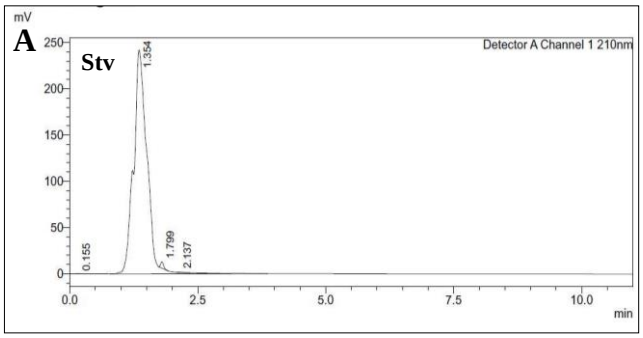
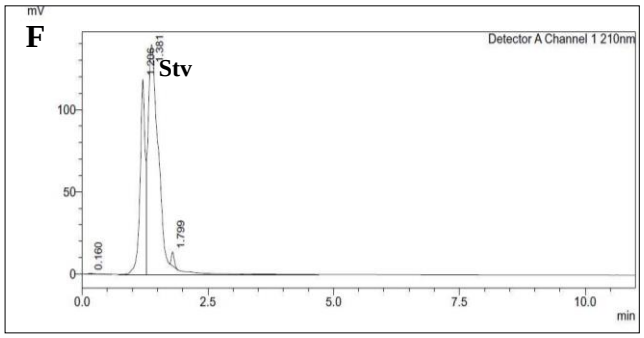


Figure 1: Chromatogram of stevioside (Stv) standard shows the peak presence at 1.383, also present in the standard are rebaudioside A (RebA), rebaudioside C (RebC) and dulcoside A (DulA)

Results of stingless bee honey samples analysis in Figure 2A-2L show the presence of stevioside by comparing the retention time in the standard (Figure 1), determined the presence of the Stevia plant compound in the stingless honey. However, there are differences on stevioside compound in stingless bee honey in concentration based on mV value (axis-y) between the months. The concentration of stingless bee honey depends on the surrounding environment and weather (Nascimento and Nascimento, 2012). On the dry seasons, such as in February to June, honey concentrations are viscous than other months especially on monsoon seasons. A study of pollen observation via microscopy approach also successfully determined the presence of *Stevia rebaudiana* pollen from the same colonies in this study (Shamsul Bari and Asma', 2015). Table 1 presents the retention time of the standard and the stingless bee honey samples.


A
Stv
Detector A Channel 1 210nm
mV
min


F
Stv
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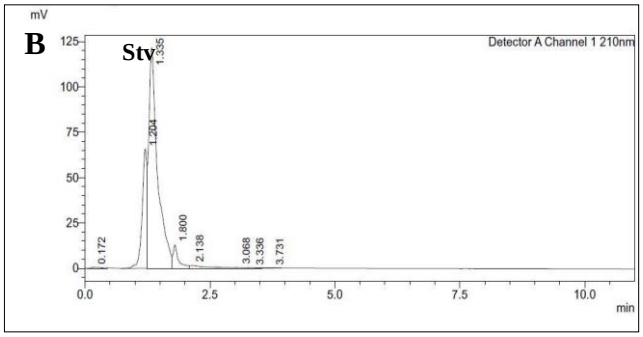
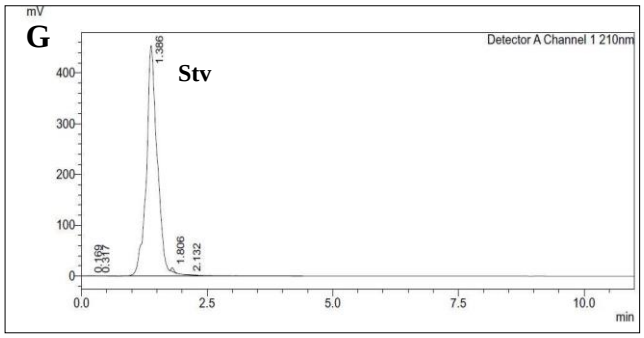
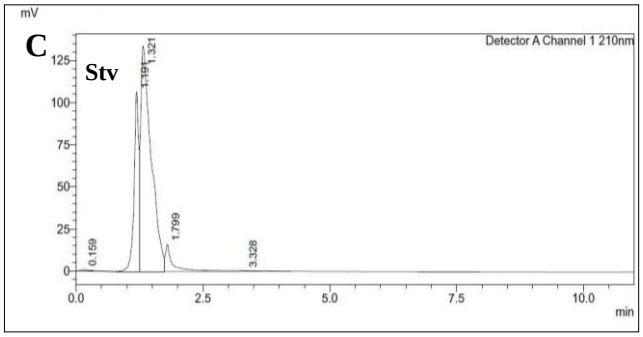
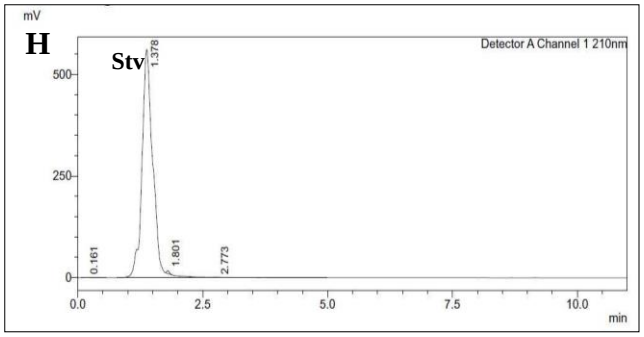

B
Stv
Detector A Channel 1 210nm
mV
min


G
Stv
Detector A Channel 1 210nm
mV
min


C
Stv
Detector A Channel 1 210nm
mV
min


H
Stv
Detector A Channel 1 210nm
mV
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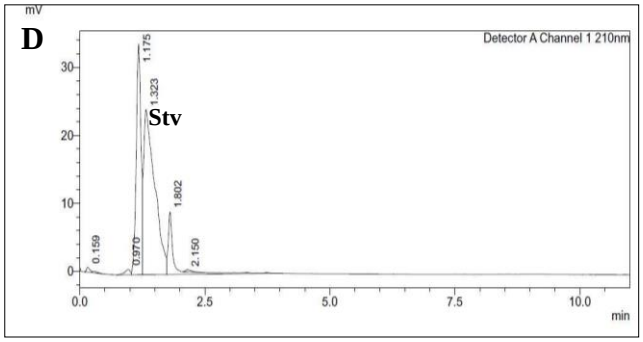
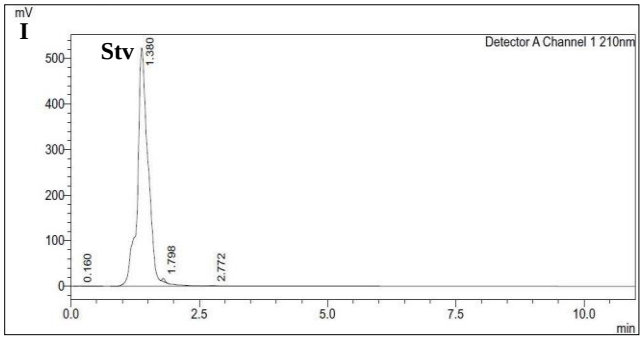

D
Stv
Detector A Channel 1 210nm
mV
min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Stv
Detector A Channel 1 210nm
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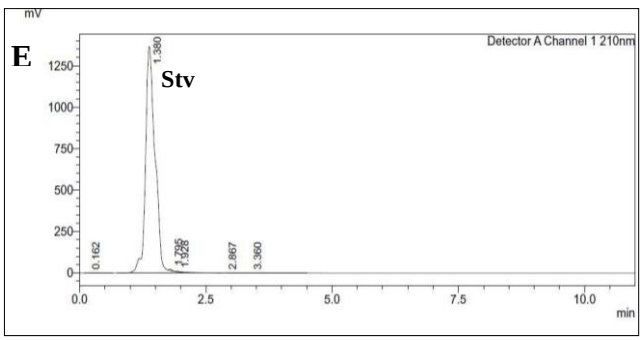
E
Stv
Detector A Channel 1 210nm
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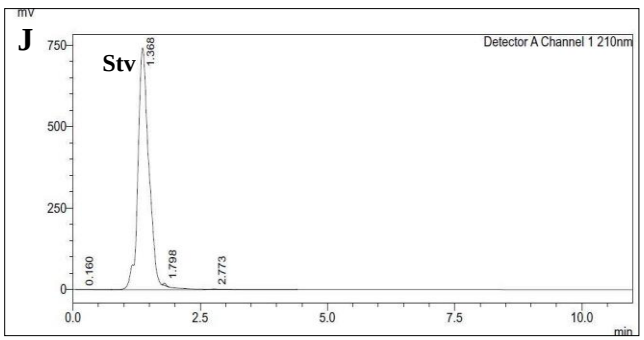
J
Stv
Detector A Channel 1 210nm
mV
min

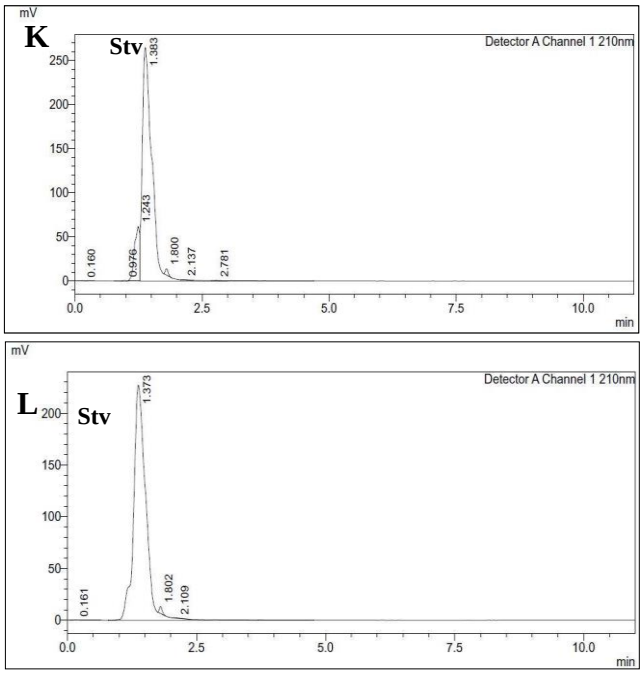

Figure-2: Chromatogram of stevioside-containing in stingless bee honey from samples collected in (A) January, (B) Febuary, (C) March, (D) April, (E) May, (F) June, (G) July, (H) August, (I) September, (J) October, (K) November and (L) December.

Table 1: Retention time of standard stevioside and stingless bee honey sample

	Retention time (R_t)
Standard Stevioside	1.383
Jan	1.354
Feb	1.335
Mac	1.321
April	1.323
May	1.380
June	1.381
July	1.386
Aug	1.378
Sept	1.380
Oct	1.368
Nov	1.383
Dec	1.373

Conclusion

In conclusion, the integration of stingless bee and stevia is successfully produced as the consistently presence of stevioside compound in the stingless bee honey, which is capable to improve the taste of stingless bee honey besides rich known health's beneficial compounds.

Acknowledgement

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Contribution of Authors

Razak SBA: Conceived idea, manuscript writing and data interpretation
Ismun A: Data interpretation
Samsuddin SA: Designed research methodology and data collection

Disclaimer: None.
Conflict of Interest: None.
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