

Aim

Using NMR combined with a multivariate statistical approach to determine what metabolites can be used to characterize honey, and at what level. (Country? Species? City?)

Background

Indigenous Australians have long claimed that native Australian honey has many health benefits. Recent publications from Mary Fletcher's group [1] have shown that Australian and Malaysian stingless bees have a unique sugar known as trehalulose. These findings support the earlier claims, as trehalulose has interesting chemical and physiological properties that set it aside from its carbohydrate counterparts. It is non-cariogenic, which means it does not promote tooth decay. And it is around 70% as sweet as glucose. [1]

More than 500 species of stingless bees are known across tropical and sub-tropical regions (Figure 1). This raises the interesting question, how different stingless bee honey is when compared between geographical regions and species?

Our research has focused on four species of stingless bees, two from Australia (*T. carbonaria*, *T. hockingsi*) and two from Malaysia (*G. thoracica*, *H. itama*) that produce honey. Using honey samples from these stingless bees, and from different geographical locations, we can attempt to answer the following questions:

- Can we characterise SB honeys?
- What are the varieties of SB honey that we can get?
- How different are SB honeys from different countries?
- Collected by different bee species and in different geographical areas?

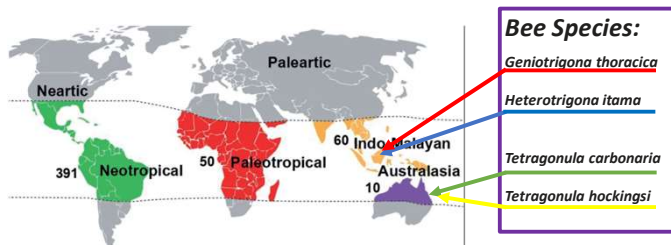
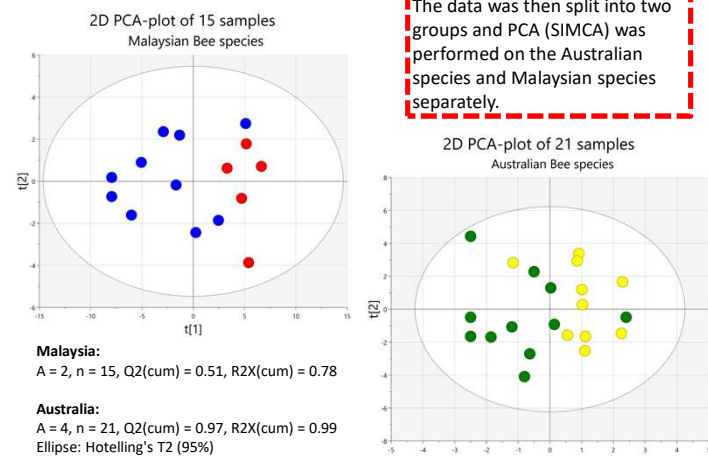
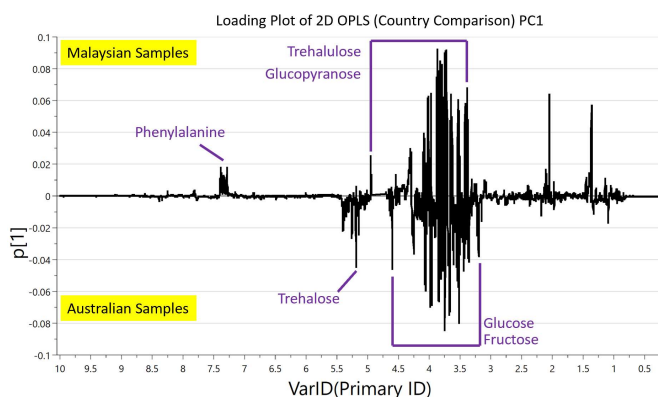


Figure 1: Geographic distribution of stingless bees [2]

Bee Species Comparison



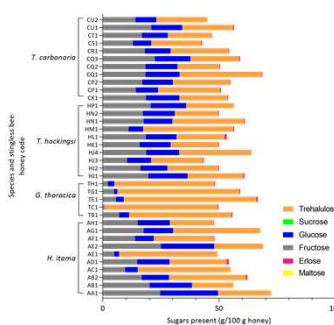
Metabolite Identification



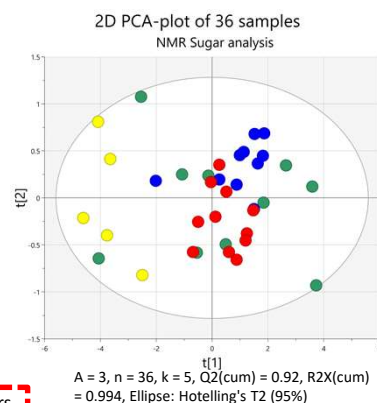
References

- [1] Fletcher, M. T., Hungerford, N. L., Webber, D., Carpinelli de Jesus, M., Zhang, J., Stone, I. S. J., Blanchfield, J. T., & Zawawi, N. (2020). Stingless bee honey, a novel source of trehalulose: a biologically active disaccharide with health benefits. *Scientific Reports*, 10(1), 12128–12128. <https://doi.org/10.1038/s41598-020-68940-0>
- [2] Rattanawanee, A., & Duangphakdee, O. (2021). Southeast Asian Meliponiculture for Sustainable Livelihood. Retrieved 15 October 2021, from <https://www.intechopen.com/chapters/70501>

Preliminary Analysis

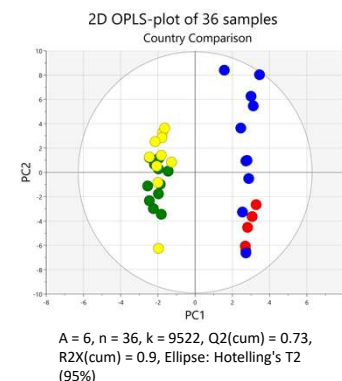


Ion chromatography was used to quantitate the sugars against sugar standards in 36 samples of stingless bee honey to assess differences between samples and between species (Mary Fletcher).

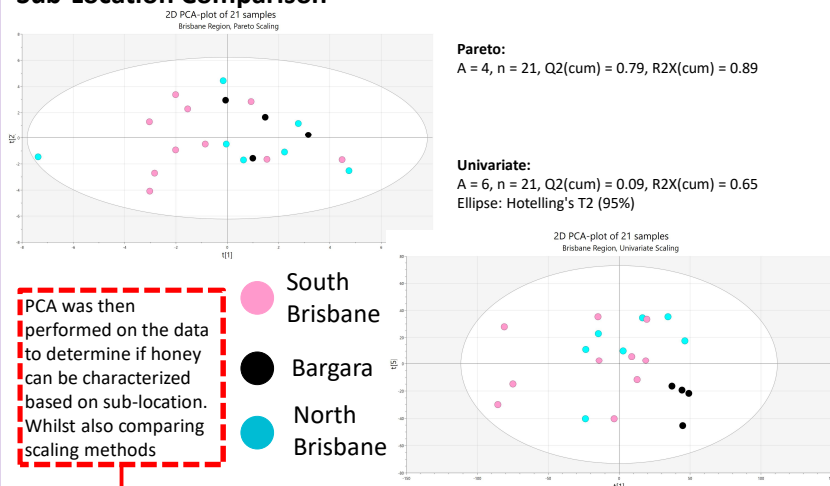


Country Comparison

The NMR Data was phase corrected (Topsin), baseline corrected and then aligned (Mnova). After binning at 0.001 ppm bucket width, spectra were normalised to total spectral intensity. OPLS was then performed to answer the question "What are the differences between the countries?" after PCA was used on preliminary sugar analysis which answered the question "Is there a difference?"



Sub-Location Comparison



Finally, Chenomx was used to identify the important metabolites that separated the country classes. This was difficult as the Chenomx library is limited and struggles to identify metabolites in samples that do not have a pH of 7

| Molecule | Description |
|---------------|---|
| Phenylalanine | Aromatic amino acid, Common in Honey |
| Trehalose | Sugar, found is SB Honey |
| Trehalulose | Sugar, found is SB Honey |
| Glucopyranose | Pyranose form of glucose, Common in Honey |
| Fructose | Simple Sugar, Common in Honey |
| Glucose | Simple Sugar, Common in Honey |

Conclusion

The Analysis of the data supports the idea that Stingless bee honey can be characterized based on metabolites identified in NMR. But, only at a country and species level. The 2D PCA of Sub-Locations was not strong enough to separate any classes. Although this is likely due to the sample size. Future research should focus on incorporating more geographical regions, such as South America or Africa, and also more Stingless Bee species.

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