Abstract
The recent discovery of the beneficial low-GI sugar disaccharide trehalulose as a major component in Australian stingless bee honey has given scientific credence to long-held claims that the honey has therapeutic properties, including antidiabetic and antioxidant benefits. However, the origin of this rare sugar has been unknown.

As part of this project, the natural abundance of trehalulose and organic acids was examined in more than 100 stingless bee honeys samples from different species and locations. The research demonstrated that native Tetragonula carbonaria bees fed sucrose (table sugar) readily produce honey rich in trehalulose. This new knowledge suggests that stingless bees that feed on sucrose-rich nectar should produce trehalulose-rich honey. Storage experiments further demonstrated that trehalulose is quite stable in honey whether the honey is stored in the fridge, at room temperature or in the hive.

This knowledge will assist industry with developing a Food Standard, with trehalulose as a distinctive marker for this premium product.

Background
Australian stingless bee honey attracts a high market value and is reported to have a range of beneficial properties, including antidiabetic and antioxidant benefits. However, no specific bioactive honey component has previously been associated with this activity. It has long been recognised that Australian stingless bee honey contains high levels of a single disaccharide, with this sugar previously reported as maltose – although this sugar was never separated or characterised.

The discovery that this disaccharide in honeys of the major Australian stingless bee species Tetragonula carbonaria and Tetragonula hockingsi is actually the more unusual trehalulose provides a specific marker for this premium honey. The finding also affords scientific credibility to claims regarding the benefits of this honey. Trehalulose has previously been shown to be both low GI and acariogenic, meaning it is tooth friendly and doesn’t cause tooth decay.

Trehalulose has not previously been found as a major component in any food. As such, the presence of trehalulose in stingless bee honey presents significant marketing opportunities for the rapidly emerging stingless bee industries in Australia and overseas.

First, however, the origin of the abundance of this rare sugar in stingless bee honey required detailed investigation. Without this knowledge, the Australian stingless bee honey industry has lacked information about what conditions maximise the level of trehalulose present.

Objectives
The principal objectives of this project were to identify conditions under which maximal bioactive trehalulose is formed in stingless bee honey, and to understand the stability of this beneficial sugar under storage conditions both inside and outside the hive. In doing so, we also sought to examine the production of organic acids in stingless bee honey under the same conditions. These components are responsible for the sour and tangy tastes associated with this honey that find favour with honey connoisseurs and specialty chefs.

A further objective was to work with keepers of stingless bee hives to provide industry understanding of the conditions required to produce stingless bee honey with optimised and standardised trehalulose content, and to assist the Australian Native Bee Association (ANBA) with developing an Australian Food Standard based on this novel disaccharide being a marker for this premium product.

Research
This project was undertaken to determine both the origin and stability of trehalulose in stingless bee honey, and also to determine the relative sugar and organic acid composition across stingless bee species, location and other hive conditions. This research combined good science with integrated industry involvement and communication to ensure uptake of project outputs to benefit this burgeoning industry. As part of the project:

1. Tetragonula carbonaria stingless bees in experimental hives were fed pure sugars (sucrose and a glucose/fructose mixture), and honey was collected and analysed for sugar composition to determine the basis of trehalulose production.

2. Replicate stingless bee honey samples were ‘aged’ both in-hive and after harvest under controlled laboratory conditions (e.g. 4 °C, 20 °C and 30 °C) to determine the impact of storage condition on the level of this important sugar.

3. More than 100 stingless bee honey samples of known provenance (stingless bee species, hive location, season, length of time in hive, hive history etc) were sourced from industry collaborators. These included honey samples from five local stingless bee species (Tetragonula carbonaria, Tetragonula hockingsi, Tetragonula denvanporti, Austroplebeia australis, Austroplebeia cassiae) from natural forest, suburban garden and farm locations. These samples were analysed for both bioactive sugar composition and also organic acid production.

Outcomes/key findings
By experimentally feeding sugar solutions to confined experimental colonies of the Australian stingless bee Tetragonula carbonaria, we have established the origin of trehalulose in stingless bee honey. We observed almost complete conversion of sucrose (table sugar) fed to stingless bees to trehalulose. In contrast, feeding a glucose/fructose (1:1) mixture did not result in trehalulose formation.

Hence, stingless bees that feed on sucrose-rich nectar should produce honey rich in trehalulose. Honey from one hive in a macadamia orchard (with reportedly sucrose-rich nectar) contained the highest trehalulose as a percentage of total sugars. However, Tetragonula hives in suburban gardens contained the highest trehalulose content by honey. Storage experiments demonstrated that trehalulose is quite stable in honey regardless of whether it is stored in the fridge, at room temperature or at higher hive temperatures.

Analysis of honeys of different provenance showed that while there is natural variation between similar honey samples, the largest determinant of sugar and organic acid composition is bee species and hive habitat. Major sugars present in honey of all Tetragonula species were fructose, glucose and trehalulose. By comparison, honey of Austroplebeia species contained fructose, glucose, some sucrose and significantly lower levels of trehalulose than that of Tetragonula species.
Honey of *Tetragonula* species contained high levels of gluconic, acetic and lactic acids; honey of *Austroplebeia* species contained similar gluconic acid levels but lower levels of acetic and lactic acids. The results and knowledge gained will enable the stingless bee honey industry to produce honey with a more consistent and reliable composition. The findings will also help facilitate the development of an Australian Food Standard for stingless bee honey, with trehalulose used as a distinctive marker compound for this premium product.

This information has been conveyed to industry and the wider community in formal presentations, written articles and informal discussions, and disseminated through industry publications, such as the ANBA newsletter *The Cross Pollinator* (May 2021 issue).

**Implications for industry**

This research has served to elucidate the origin and stability of the beneficial sugar trehalulose in Australian stingless bee honey. The presence of this distinctive sugar with known low glycaemic index provides a ready and valuable authenticity marker for stingless bee honey, to guard against substitution and/or adulteration with honey bee honey or sugar-based syrups.

Outputs of this research include establishing the stability of this disaccharide under normal storage conditions, and also analysis of the concentration range and profile of sugars and organic acids present in honeys across different species and situations. This information will lead to the development of a more standardised stingless bee honey product.

This research has been publicised in both scientific and popular media, and will foster consumer awareness of the beneficial value of stingless bee honey – and potentially increased consumer demand for this high-value food product. Australia has a burgeoning health food industry based on native food products, and Australian stingless bee honey has a ready but as yet not fully exploited market within this sector.

Results of this research and associated scientific publications will provide valuable scientific data for the Australian Native Bee Association in its quest for an Australian Food Standard for stingless bee honey.

**Publications**


**Industry resources**


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