RESEARCH/BUSINESS

C4 SUGARS: ANSWERS TO COMMONLY ASKED QUESTIONS

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At Analytica Laboratories, we have processed a large number of honey samples for C4 sugars over the past three years and have found a set of recurring questions that are asked when a test result comes back near to or over the threshold level of 7% C4 sugars. In this article, I will endeavour to answer these commonly asked questions and shed some light on current research and methodology to identify genuine sugar adulteration in mānuka honey.

Background

The demand for C4 sugar testing has increased over the past few years, with many buyers requiring beekeepers to have C4 sugar testing carried out on their drums before a sale will proceed. This reflects the state of international markets, where adulteration of honey with sugar has led to increasing interest by regulators and consumer groups in detecting adulteration.

False positive results for mānuka honey

Mānuka honey continues to face scrutiny with the ongoing risk of high-grade (high MG) honey failing the AOAC 998.12 C4 sugar test for sugar adulteration. Evidence has been found of an anomaly in the C4 sugar test that seems peculiar to mānuka, resulting in false positive C4 sugar results. Despite this evidence, it is important to note that beekeeping practices such as the use of cane sugar as supplementary feed, can—and is more likely to—cause honey to fail a C4 sugar test. This is because when these C4 sugars (such as cane sugar) make their way into the honey, the honey can genuinely contain too much C4 sugar!

What is the difference between the C4 AOAC and C4 Screen methods, and when should I use them?

Sugars from tropical plants like sugar cane and maize/corn are produced using a photosynthetic pathway referred to as the C4 pathway. Nectar, which is collected by bees, comes from plants that use a different process of photosynthesis referred to as the C3 pathway.

The AOAC 998.12 C4 sugar method is an internationally recognised method for determining the concentration of C4 sugars in honey. The AOAC C4 sugar test is recommended for any final production batches of honey, especially where the honey is being exported to a country that enforces C4 sugar requirements.

The C4 Sugar Screen test is a simplified version of the AOAC test providing a cheap and effective alternative to the full AOAC method. Although this method is cheaper, it is important to note that it also produces results that are more variable. For this reason, the C4 Screen test is more suitable where accuracy is not so crucial, such as testing drums at extraction, or for buying and selling.

For more information about the different C4 options, refer to the article by Dr Anatoly Chernyshev: The C4 sugar test: AOAC vs. screening method, *The New Zealand BeeKeeper*, August 2017, pages 11–13.

I've had hives in pasture and hives in mānuka handled in the exact same way, but my honey from the mānuka crop has higher C4 sugar levels, why?

Extensive work carried out by Dr Karyne Rogers (GNS Science) has confirmed that high MG mānuka is prone to false-positive results when tested using the AOAC 998.12 C4 sugar test (Rogers et al., 2014; Rogers, Grainger, & Manley-Harris, 2014; Rogers, Somerton, Rogers, & Cox, 2010) . This means it is possible that a hive containing high MG mānuka honey could produce higher C4 sugar results compared to a hive producing pasture honey when handled in the same way.

Despite the known issues with mānuka and C4 sugars, it is important not to assume that because a honey is mānuka the C4 sugar results must be false. Feeding is one of the major contributors to genuine C4 sugar fails in mānuka and non-mānuka honey (Rogers et

al., 2014). The C4 Sugar AOAC and Screen tests rely on carbon ratios measured in sugars and proteins found in the honey. Supplementary sugars or proteins fed to bees prior to, or during nectar flow can contribute to C4 sugar fails, so it is important to avoid over-feeding, and honey should not be extracted from the brood box (Rogers et al., 2014). It is perhaps too commonly assumed that C4 sugar results are high because the honey is mānuka when in reality, genuine C4 sugars have been introduced to the honey by over-feeding bees too close to nectar flow or to prevent hive starvation during a poor season.

Will my C4 sugar results change over time?

It is known that in non-mānuka honeys such as clover, C4 sugar values tend to be stable and do not change over time (Rogers, Grainger, & Manley-Harris, 2014). This means a C4 sugar result at extraction should be constant in the honey after heating and storage. For this reason, the C4 AOAC test is well suited to the vast majority of non-mānuka honey varieties where a positive result demonstrates the genuine presence of C4 sugars in the honey.

The same cannot be said for C4 sugar values in mānuka honey over time. It has been demonstrated that as mānuka honey ages (or is exposed to heat), the apparent C4 sugar levels increase over time. Research by Rogers, Grainger, and Manley-Harris (2014) demonstrated that some active mānuka honeys showed an apparent increase in C4 sugars of up to 280% with heat and storage. Although there seems to be a correlation with the level of MG in the honey, it is not well understood what the exact cause of this C4 sugar change in mānuka honey is. While significant research is being carried out to identify the cause of this change, it is currently not easy to explain exactly why there is this unique shift in mānuka honey.

Can I use a sugar profile to prove that my mānuka is unadulterated?

As honey adulterators get smarter, laboratories around the world are needing to develop more sophisticated methods to catch them. Several methods are emerging that may provide a reliable solution for New Zealand honey.

Sugar profile testing exists in the CODEX standard for honey as a method for detecting specific sugars in honey including glucose, fructose and sucrose (Codex Alimentarius Commission, 2001). Knowing the major sugars in mānuka honey are glucose and fructose (Alvarez-Suarez, Gasparrini, Forbes-Hernández, Mazzoni, & Giampieri, 2014) and following CODEX, it is expected that honey should have no less than 60% fructose and glucose content (Codex Alimentarius Commission, 2001). Addition of artificial sugars could skew this sugar profile and point to adulteration.

Another set of methods that have emerged in recent times test for unique chemical markers found only in specific sugar types; for example, in rice syrup or beet sugars. Examples of these include the SM-R, SM-B and SM-X methods that have emerged from China (Unique Mānuka Factor Honey Association, 2016).

Another alternative method for detecting sugar adulteration is NMR (Nuclear Mass Resonance) based testing, which uses unique molecular structures in honey to identify any irregularities such as foreign sugars (Spiteri et al., 2015). Currently there is no appropriate NMR and associated library of samples in New Zealand capable of doing this test effectively.

Will the C4 sugar limit be changed from 7% to a higher value?

The AOAC 998.12 method for honey was revised in 2013 to allow for a broader interpretation of test results by considering the δ13C value of the honey (a component of the carbon isotope analysis in the AOAC method) in conjunction with the 7% C4 sugar limit. As this revision doesn't specifically refer to mānuka, there is still some uncertainty about how accurately the AOAC method represents the true C4 sugar levels in mānuka honey.

In the absence of any further revision of the existing AOAC method, alternative methods are being evaluated to test for sugar adulteration in mānuka honey (as mentioned in more detail above).

Intensive work is continuing in this area within New Zealand and abroad.

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