

RESEARCH

C4 SUGAR ANALYSIS

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Introduction

C4 sugar analysis of honey is an internationally recognised way of testing whether the honey has been adulterated with other sugars. Unfortunately, recent experience is that manuka honeys can fail the test without any sugar adulteration, and there is ongoing work taking place to understand why.

What is C4 sugar analysis and why should we test for it?

The CODEX standard for honey states that honey sold should not have anything added to or removed from it (Codex Alimentarius Commission, 2001). However, with honey being a valuable product, many countries are concerned that honey offered for sale may have been adulterated by the addition of low-cost sugar (cane sugar; high fructose corn syrup) to increase the amount of 'honey' available to sell. C4 sugar testing is used to identify if sugar has been added to honey, based on an internationally accepted standard method called AOAC 998.12 (AOAC International, 1999).

The C4 sugar test analyses the ratio of the two most common isotopes of carbon (^{12}C and ^{13}C) in both the whole honey and the protein which is precipitated out of the honey using isotope ratio mass spectroscopy (IRMS).

The ratio of $^{13}\text{C}/^{12}\text{C}$ is different in honey produced from nectar (which comes from C3 plants) compared to cane sugar or high fructose corn syrup (which come from C4 plants). The basic principle of the C4 sugar test is that if honey has not been adulterated with additional sugar, the ratio of $^{13}\text{C}/^{12}\text{C}$ in the whole honey and the protein precipitated from that honey will be very close. However, if sugar has been added, the ratio of $^{13}\text{C}/^{12}\text{C}$ will be different in the whole honey and the protein. Internationally it is accepted that the difference in the ratio of $^{13}\text{C}/^{12}\text{C}$ between whole honey and protein (also called $\delta^{13}\text{C}$) in unadulterated honey will be less than or equal to 7%.

Honey from hives that have been fed sugar for management reasons may fail a C4 sugar test, especially if harvested early in the season. It is best to test individual batches before blending to avoid contamination of a large blend of honey. Unfortunately, unlike tutin, composing

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of samples in the laboratory can't be used to provide an indication of the likely C4 sugar test result of the individual batches.

Complications for manuka honey

Between 2010 and 2012, over \$6 million of New Zealand honey exports failed the C4 test (Rogers, 2014). Further investigation found that manuka honey which had not been adulterated was susceptible to failing the test. While honey usually fails the test because there is a positive shift in the $\delta^{13}\text{C}$ honey (due to addition of C4 sugar), manuka honey fails due to a negative shift in the $\delta^{13}\text{C}$ protein.

Figure 1 shows the results of 765 honey samples tested for C4 sugar at Analytica Laboratories in early 2015; 43.5% exceeded

the threshold of 7% and failed the test. While these samples were from a range of floral origins, there will have been an emphasis on manuka honeys.

It is currently unknown what causes the negative shift in $\delta^{13}\text{C}$ protein of manuka honey, and it is an area of active research. Various hypotheses have been or are being investigated, such as the contribution of pollen (Rogers, Somerton, Rogers, & Cox, 2010) and the effect of dihydroxyacetone (DHA) and methylglyoxal (MG) in the honey. Research in 2014 (Rogers, Grainger, & Manley-Harris) showed an increase in the %C4 over time for manuka honeys, as well as clover honeys spiked with DHA, compared to no change in clover honeys stored under the same conditions. Figure 2 shows the change in C4% over time for a manuka honey stored at 37°C.

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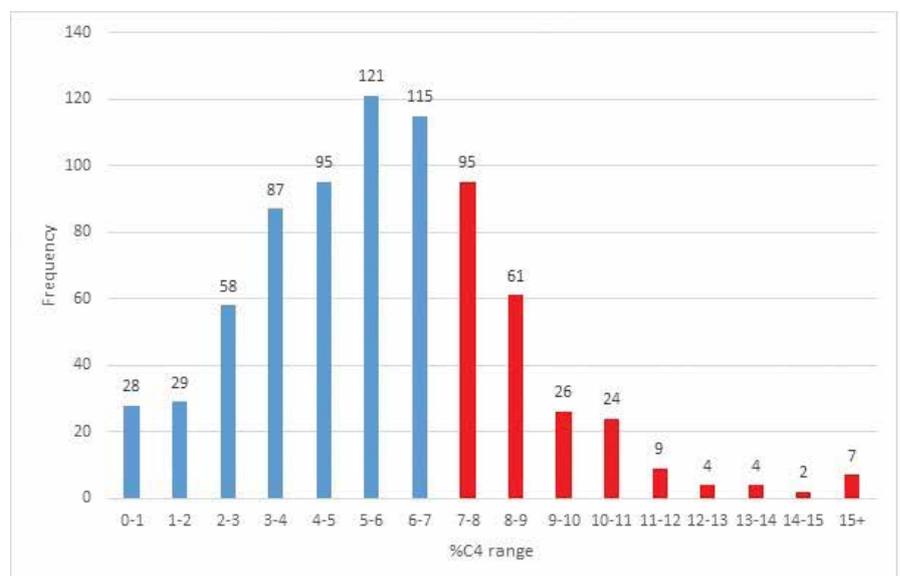


Figure 1. Distribution of %C4 for honey samples of various floral types analysed by Analytica Laboratories. Samples above 7% fail the C4 sugar test.

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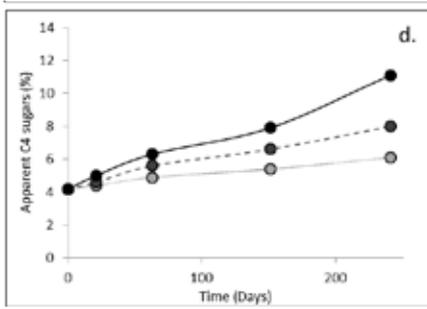


Figure 2. Apparent C4 sugar contents (%) of a manuka honey (Rogers, Grainger, & Manley-Harris, 2014).

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What are C3 and C4 sugars?

There are three ways in nature that carbon can be incorporated into plants through photosynthesis; two of these pathways are the Calvin cycle (also known as the C3 cycle) and Hatch-Slack cycle (the C4 cycle). Nectar is produced by plants using the C3 photosynthetic cycle while sugars like cane sugar (from sugar cane) and high fructose corn syrup (from maize) are produced using the C4 cycle. Carbon naturally exists as two different stable isotopes, which behave in the same way but have slightly different molecular weights. Carbon-12 (¹²C) is the most abundant in nature (98.89%); and Carbon-13 (¹³C) is far less common (1.1%) and is heavier than ¹²C. More ¹³C is incorporated into sugar produced in the C4 cycle, and this can be analysed by isotope ratio mass spectroscopy (IRMS).

Conclusion

C4 sugar testing is an understandable response to the global problem of adulteration of honey. While New Zealand exporters can have their honey tested to satisfy the requirements of overseas customers, there is ongoing work taking place to understand the reasons why manuka honey produces unexpected results in this test.

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