RESEARCH

APPARENT C4 SUGARS IN MĀNUKA HONEYS

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The C4 sugar test is a general qualification procedure for marketed honey. Current standards limit the amount of C4 sugars in honey to 7%. Most floral types of honey produce stable C4 sugar test results over the period of their shelf life. High-quality mānuka honeys were shown to inflate their C4 sugar content when stored, whereby significantly devaluing the product. This effect is exacerbated by the practice of 'ripening' fresh honeys, especially by heating. We at Analytica did a thorough investigation on that phenomenon and came up with a kinetic model, which reliably forecasts the change in apparent C4 sugar in mānuka honeys.

Apparent C4 sugars

C4 sugars are the major commercial sugars produced by particular plants (C4 plants). The latter include, for example, sugar cane and corn. All melliferous plants are C3 plants. While the sucrose sourced from these different plant groups is the same chemically and by taste, it does differ in the content of ^{13}C isotope of carbon (the value expressed as $\delta^{13}C$ thereafter). This serves as a ground for the instrumental analysis of C4 sugars.

To test for C4 sugars, one needs to separate the protein fraction (approximately 1% by mass) of honey, and analyse both the protein and bulk honey for ^{13}C. The percentage of C4 sugars is calculated by the formula below.

$$\%C4\ sugars = \frac{\delta^{13}C_{protein} - \delta^{13}C_{honey}}{\delta^{13}C_{protein} - (-9.7)} \cdot 100\%$$

The -9.7 value is the consensus average $\delta^{13}C$ of known C4 adulterants. According to this formula, when the isotopic composition of bulk honey and protein is the same (usually around -26), there are zero C4 sugars.

The addition of C4 sugars causes $\delta^{13}C$ of the honey to shift towards higher (more positive) values, which will be discovered during testing.

The term 'apparent C4 sugars' refers to the cases when the $\delta^{13}C$ for honey remains normal, but the $\delta^{13}C$ for protein shifts to more negative values due to (yet) unknown reasons.

In high-grade mānuka honeys, this kind of shift was observed independently by several laboratories. It has been found that $\delta^{13}C$ for protein might change in time and this is accelerated at higher temperatures; for example, during postharvest ripening of honey. During this process, the total increase in the percentage of C4 sugars could get as high as 8%.

Ripening honey is kept for several months until it achieves the desired level of non-peroxide activity (NPA), the major factor determining the market price of honey. Since this kind of incubation leads to the increase in apparent C4 sugars too, there were numerous occasions when batches of mānuka honey were deemed unsuitable for export as a result.

A previous publication (Rogers, Grainger & Manley-Harris, 2014) indicated that such kind of shift could have been caused by dihydroxyacetone (DHA) and/or methylglyoxal (MG) binding to the protein. However, these findings were questionable, because both DHA and MG are synthesized in the same biochemical cycle as other sugars. Therefore, they should have a similar $\delta^{13}C$ and are unable to distort the isotopic composition of the protein significantly.

In order to understand the mechanism of the shift in $\delta^{13}C$ of the protein, Analytica, with funding from the UMF™ Honey Association (UMFHA), carries out a dedicated incubation study of eight different varieties of honey. The set of honeys included a variety of mānuka honeys, clover honeys, and polyfloral honeys. To track the fate of dihydroxyacetone (DHA), we added a small amount of DHA labelled with a radioactive ^{14}C isotope to all samples.

Incubating these honeys at 27°C over a period of 18 months, we regularly analysed them for DHA, MG, C4 sugars, and the accumulation of ^{14}C label in the precipitated protein.

Three pivotal observations were made during this experiment:

(i) the percentage of apparent C4 sugars changes significantly only in fresh mānuka honeys

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(ii) the rate of this change does not depend on the concentration of DHA and MG

(iii) the binding of DHA and MG to the protein is high (30–40% of their total concentration) and does not depend on the incubation time.

These findings indicate that there might be a yet-unknown substance X in mānuka honey, which is binding to the protein over time and has very negative $\delta^{13}C$. Despite the still-enigmatic nature of this substance, the accumulated data have allowed us to establish a kinetic model to forecast the change in apparent C4 sugars.

Analytica's kinetic model

The model is based on our data combined with the data from the previous study (Rogers, Grainger, & Manley-Harris, 2014). Both datasets were in good agreement with each other and let us cover a wider temperature range when testing the model against experimentally measured C4 sugar values (Figure 1).

We were forecasting the evolution of apparent sugars at five different temperatures, based solely on the analyses at day 0 (Figure 1). Since we also did experimental measurements at various time points, it is possible to calculate the absolute error of the forecast (plotted on the vertical axis), and compare it with the uncertainty of our C4 screening test (±1.8%).

As one can see, the forecast doesn't work for high temperatures (34°C and 37°C). However, at 27°C and below, it produces the results well within the uncertainty of the actual analytical test, at least for the term of the experiment (18 months). Playing conservatively, we would recommend using this model for the temperatures up to 25°C, which is sufficient for most honey processors.

The apparent C4 sugar forecasting tool will not only help us in the future with ongoing research, but also be a valuable aid to honey exporters who want some assurance that their quality mānuka honey will not fail the C4 sugar test in overseas markets unexpectedly. This forecasting model shall be available to our clients in the nearest future.

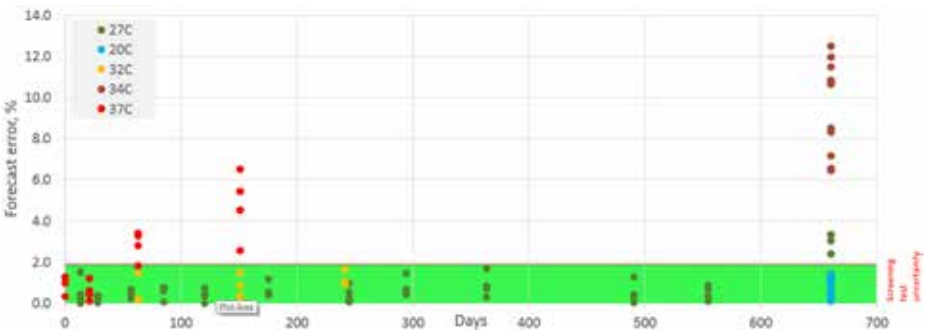

Figure 1. The model's forecast error at different temperatures and incubation times, compared to the standard uncertainty of the screening C4 sugar test (green area).

Reference

Rogers, K. M., Grainger, M., & Manley-Harris, M. (2014). The unique mānuka effect: why New Zealand mānuka honey fails the AOAC 998.12 C-4 sugar method. *Journal of Agriculture and Food Chemistry, 62*(12), 2615–2622.

[Editor's note: this is the second part of a two-part article. Part one (AOAC C4 sugar tests: the final frontier?) was published in the October 2018 journal.]