WHITE PAPER

IEH Analytical Laboratories

AOAC Official Method 998.12
Detecting C-4 Plant Sugars in Honey

Honey is a widely consumed natural food known for its nutritional value and health benefits. In addition to being a sweetener, honey is recognized for its medicinal properties, including anti-inflammatory, anti-bacterial, antioxidant, and even cancer-preventive effects (Afrin et al., 2020). As demand for honey continues to grow, especially in the United States where over 70% of honey is imported (USDA, 2022), so does the risk of adulteration.

Unfortunately, some imported honey is found to be altered — either by mixing it with cheaper sugar syrups, like corn syrup, or by feeding bees sugar syrup to boost yield. Both practices are considered unethical. In fact, the FDA found that about 10% of imported honey was violative in a recent assessment (FDA, 2021–2022).

Bees collect their nectar mainly from the flowers of C-3 plants and seldom form the flowers of C-4 plants (Cane and Corn). C-3 and C-4 plant sugars have distinct $\delta^{13}C$ signatures. $\delta^{13}C$ signature of honey and its protein fraction can help us identify the C-4 adulteration.

To detect such adulteration, we use a well-established technique called Stable Isotope Ratio Analysis for Carbon (SIRA Carbon), as described in the AOAC 998.12 method. This approach can identify whether honey contains more than 7% of C-4 plant sugars, such as those derived from corn or cane sugar.

Approach

We determine the carbon isotope ratios ($\delta^{13}C$) of both the honey and its protein fraction, which is isolated through the repetitive washing method specified in AOAC 998.12. These measurements are performed using an Isotope Ratio Mass Spectrometer (IRMS, Finnigan MAT252) coupled to an Elemental Analyzer (EA, Carlo Erba NC 1500).

By comparing the $\delta^{13}C$ values of the whole honey and its protein, we can estimate the percentage of C-4 sugar adulteration. A significant difference between these values indicates the presence of added sugars from C-4 plants, helping us ensure the integrity and authenticity of the honey. The equation that AOAC 998.12 method uses to evaluate the authenticity of honey is:

$$\text{C-4 sugars, \%} = \{(\delta^{13}CP - \delta^{13}CH) / (\delta^{13}CP - (-9.7))\} * 100$$

Where $\delta^{13}CP$ and $\delta^{13}CH$ are $\delta^{13}C$ values, ‰, for protein and honey, respectively. -9.7 is the average $\delta^{13}C$ value for corn syrup, ‰. According to the method, we report negative values from the calculations as 0% sugar. Product is considered to contain significant C-4 sugars (primarily corn and cane) only at or above a value of 7% sugars.

To establish this method in our lab, we adulterated pure honey with pure corn syrup at different percentages starting from 1 to 20%. We extracted the proteins from those adulterated bulk honeys and analyzed them to evaluate AOAC official method 998.12.

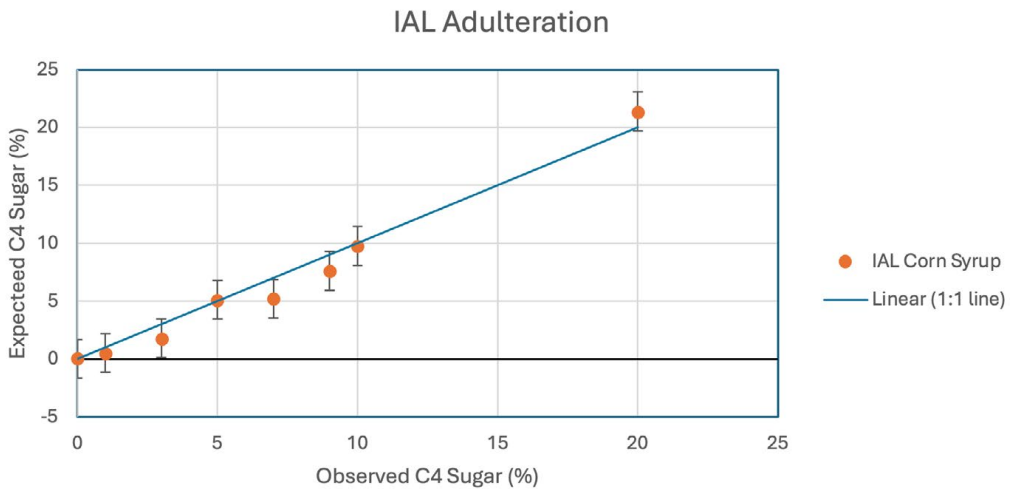
Results

We first evaluated honey authenticity using the known adulterant values. The pure corn syrup that we used as an adulterant had a value of -11.045‰ for $\delta^{13}C$, so we substituted the -9.7‰ average C4 value with our value. In an ideal scenario, the $\delta^{13}C$ of bulk honey and its protein is assumed to be the same. We took this assumption and calculated the % sugars in our lab adulterated honeys. The results are shown in table 1.

Table 1.

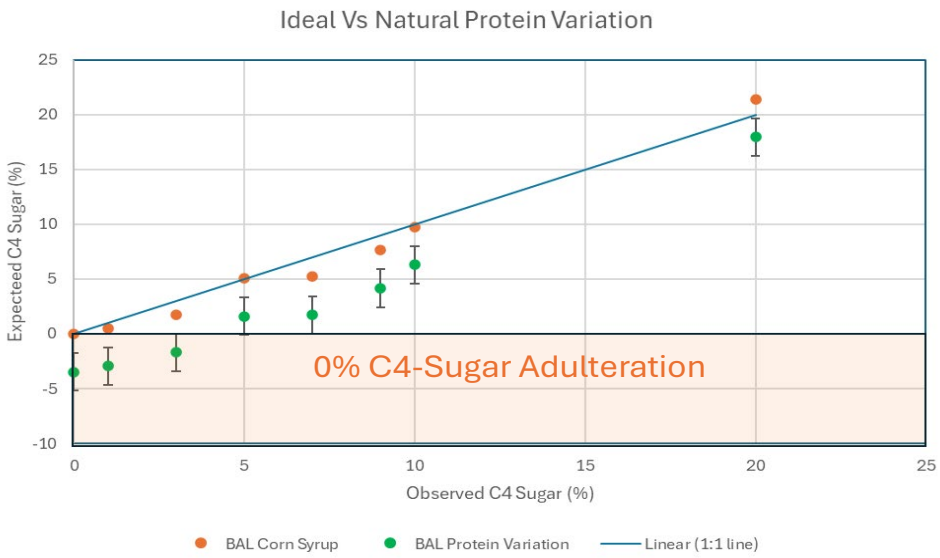
Expected C4-sugar (%)	Honey d13C vs VPDB (permil)	Protein d13C vs VPDB (permil)	Observed C4-sugar (%)	$\delta^{13}CP - \delta^{13}CH$
0	-26.57	-26.05	0.00	0.52
1	-26.57	-26.13	0.50	0.45
3	-26.31	-26.05	1.77	0.25
5	-25.65	-25.88	5.09	-0.24
7	-25.71	-25.97	5.19	-0.25
9	-25.41	-26.03	7.61	-0.62
10	-25.12	-26.07	9.75	-0.95
20	-23.47	-26.18	21.37	-2.72

Fig. 1 shows the observed C4 sugar vs. expected C4 sugar for known adulterant.



In a real-world scenario, natural variation in honey and its protein is observed between 0-1‰. Honey that we used for our adulteration analysis had a difference ($\delta^{13}CP - \delta^{13}CH$) of 0.52‰.

Fig. 2 shows results when we incorporate the natural variation between bulk honey and its protein using a known C4 adulterant $\delta^{13}C$ value.



In a scenario when we don't know the adulterant, we will be using -9.7‰ average corn syrup value for the %sugar calculation.

Fig. 3 shows the comparison between known and unknown C4 adulterant using the natural variation in bulk honey and its protein $\delta^{13}C$ values.

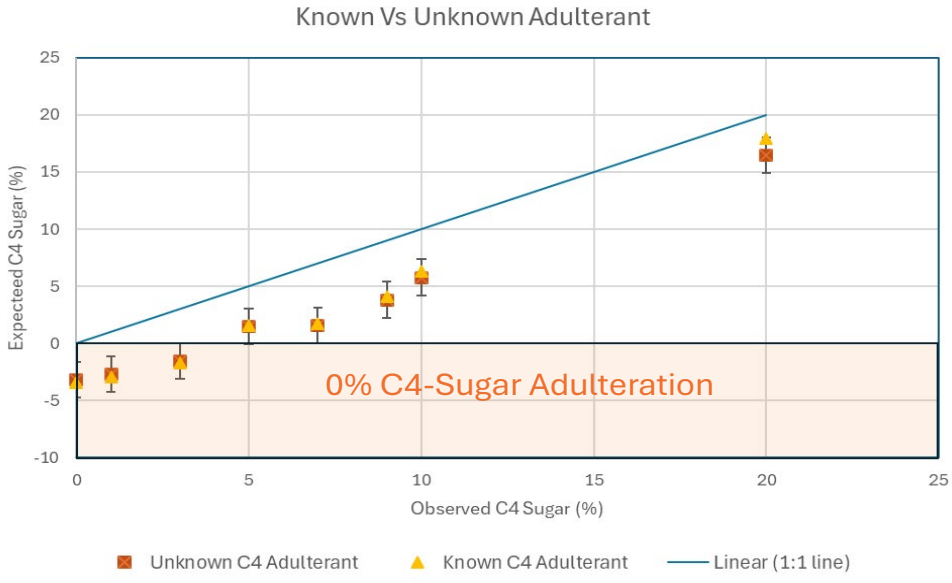
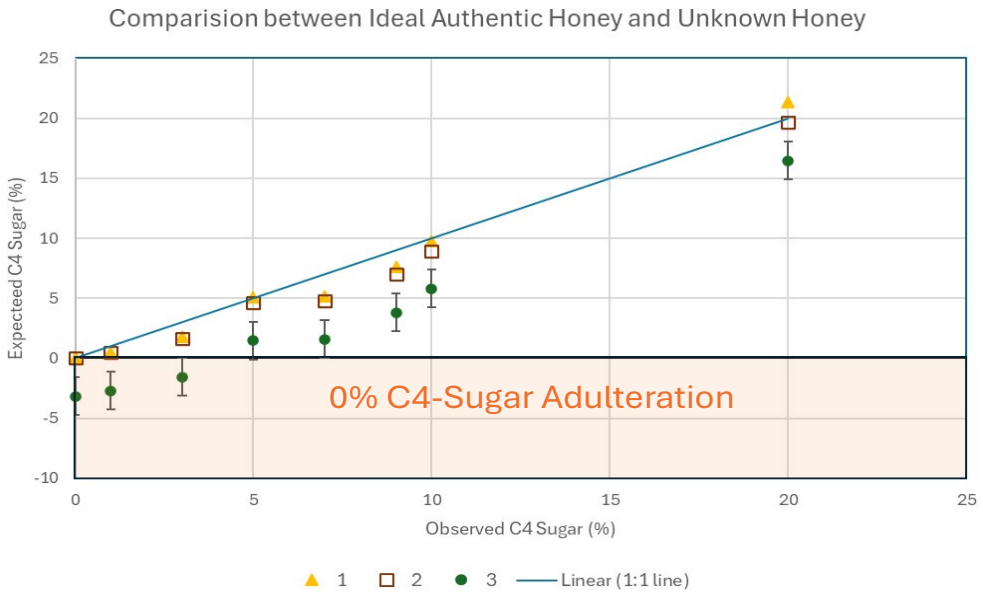


Fig. 4 shows the comparison between:

1) an ideal authentic honey adulteration
2) honey with an unknown adulterant but known natural variation
3) honey with unknown adulterant and unknown natural variation ($\delta^{13}CP - \delta^{13}CH$) between bulk honey and its protein.



To Learn more about Stable Isotope Ratio Analysis, or how we can help with your food adulteration testing needs, please contact us:

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