WHY DOES NEW ZEALAND HONEY FAIL AOAC 998.12 C-4 SUGAR ADULTERATION TESTS?

K. Rogers, S. Stewart, R. Pyne, C. Douance, J. Cooper, A. Phillips, P. Rogers
National Isotope Centre, GNS Science
Lower Hutt, New Zealand
k.rogers@gns.cri.nz
New Zealand honey problem!

Some New Zealand honey has a tendency to fail the international AOAC method 998.12 to detect C-4 sugar adulteration even though it is genuine

......especially Manuka Honey
Carbon stable isotope ratio detects differences between honey and C4 sugars.
What is AOAC method 998.12?

- Detects the presence of added cane or corn sugars (C-4 sugars) using carbon stable isotope ratio
- The method determines carbon isotope comparison of honey and its internal protein

C-4 sugar content (%) is calculated by

\[ \text{C-4 sugars} = \frac{({}^{13}\text{C}_{\text{protein}} - {}^{13}\text{C}_{\text{honey}})}{{}^{13}\text{C}_{\text{protein}} + 9.7} \times 100 \]
Unadulterated honey has a honey isotope closely related to the protein isotope.

Honey: -25 ‰  
Protein: -26 ‰  
C-4 Sugar: -10 ‰  

Pass occurs when Honey and Protein diff <1‰.
Addition of C4 sugar shifts the honey isotope in a positive direction, but not the protein isotope.

Honey: -24 ‰
Protein: -26 ‰
C-4 Sugar: -10 ‰

Pass occurs when Honey and Protein diff < 1‰
Fail occurs when Honey and Protein diff > 1‰
Pass occurs when Honey and Protein diff < 1‰

Fail occurs when Honey and Protein diff > 1‰

False positive occurs when Honey and Protein diff > 1‰

False positive results shift the protein isotope in a negative direction, but not the honey isotope.
Is the test flawed for NZ honey?

33% samples failed in 2012 (n=1004)

7% C4 sugar limit

\[ R^2 = 0.81 \]

%C4 Sugar

\( \delta^{13}C \) Honey isotopes (‰)

Pass

Fail

Apimondia, Kyiv, Ukraine  29 September – 4 October 2013
Which honeys fail....

- Manuka
- Ling or heather honey
- Tawari
- Kamahi
- Rewarewa
Mono floral honey – Tawari, Pohutukawa, Thyme, Ling or Heather, Rata

Apparent C4 Sugar (%) vs. Honey Carbon Isotope (per mil)

- Tawari
- Pohutukawa
- Thyme
- Ling
- Rata

7% C4 sugar limit
Mono floral honey – Clover, Kamahi, Rewarewa

Honey Carbon Isotope (per mil)

Apparent C4 Sugar (%)

7% C4 sugar limit
Why does honey fail the AOAC Method?

1. Excess cane sugar feeding
2. Excess protein feeding
3. Brood box extraction
4. Pollen Contamination
Why does honey fail the AOAC Method?

1. Excess cane sugar feeding
2. Excess protein feeding
3. Brood box extraction
4. Pollen contamination

Or some other unknown mechanism?
International ring test to solve this problem was unsuccessful

Removing pollen prior to protein extraction did not solve the problem for all genuine products

Manuka honey (n=676)

42% samples failed

7% C4 sugar limit

\[
\begin{align*}
\text{Apparent C-4 Sugar (\%)} & \\
\delta^{13}\text{C Honey (‰)} & \\
\end{align*}
\]
Where is the honey that always passes?

Honey Stats
676 manuka honey samples
- 284 honey samples fail 42%
- 392 honey samples pass 57%

All manuka honey passes when isotope values <-25.8‰

Genuine passes

Sample numbers

Carbon isotopes of Honey

C4 sugar fail

c4 sugar pass

Honey

Apimondia, Kyiv, Ukraine  29 September – 4 October 2013
Where is the honey that always fails?

**Honey Stats**
676 manuka honey samples
- 284 honey samples fail 42%
- 392 honey samples pass 57%

All manuka honey fails when isotope values >-24.7‰
Where are the false positive honey fails?

**Honey Stats**
676 manuka honey samples
- 284 honey samples fail 42%
- 392 honey samples pass 57%

Manuka has false positive fails when isotope values $<-24.7\%$
Low non peroxide activity (NPA) honey has less than 10% fail rate.
Low non peroxide activity (NPA) honey has less than 10% fail rate
Medium non peroxide activity (NPA) honey has 60% fail rate.
Medium non peroxide activity (NPA) honey has 60% fail rate
High non peroxide activity (NPA) honey has more than 80% fail rate.
High non peroxide activity (NPA) honey has more than 80% fail rate
The Manuka effect

• How does C-4 sugar content vary as dihydroxyacetone (DHA) converts to methylglyoxal (MG)?

• Fresh manuka honey was heated at 27, 32 and 37°C for up to 241 days

• Analyse for DHA, MG, HMF and C4 sugars

Honey heated at 37°C for 0, 63 and 241 days

Thanks to Merilyn Manley-Harris & Megan Grainger, Waikato University, and Kevin Gibbs
Heating a lower DHA Manuka for 241 days increases apparent C-4 sugar content by up to 6%

Rogers, Grainger and Manley-Harris 2013. J. Ag. Food Chem. (submitted)
Heating a higher DHA Manuka for 241 days increases apparent C-4 sugar content by up to 9%.

Rogers, Grainger and Manley-Harris 2013. J. Ag. Food Chem. (submitted)
The new Manuka honey challenge: Post harvest storage is critical

- As NPA Manuka honey grows in activity, apparent C-4 sugars also grow.
- This is caused by amino acid consumption in the protein during the DHA to MG conversion.
- The net effect changes the protein isotope, giving a larger estimate of ‘apparent’ C-4 sugar in Manuka honey, especially when NPA >10.
- Heat and storage time accelerate this reaction.
When manuka honey isotope $<-24.7\%$, apparent C-4 sugar threshold is up to 13\%.
C-4 sugar limit for manuka honey is raised to 13% when genuine honey isotopes < -24.7‰

Apimondia, Kyiv, Ukraine  29 September – 4 October 2013

GNS Science
Fail rate for Manuka honey is reduced from 43% to 19% and false positive results are eliminated.
Recommendations

• AOAC 998.12 method is an acceptable method to identify illicit C-4 sugar addition in most honey

*Pure honey (free of corn or cane sugars) generally yields a value of C-4 sugars of ≤7%*

• However an exemption is required for New Zealand manuka honey

*New Zealand manuka honey with C-4 sugars exceeding 7% and up to 13% are considered genuine provided the honey isotope value is more negative than -24.7 ‰*
Thanks to all those involved...

The Funders

• Ministry for Primary Industries
• NZ Honey Packers and Exporters Ass.
• Manuka Health NZ Ltd
• Honey New Zealand
• The Honey Company Ltd
• AGMARDT
• Ministry of Foreign Affairs & Trade
• Honey Trust
• National Beekeepers Assoc.
• Federated Farmers Bee Industry Group
• Bee Product Standards Council
• Claridge’s Organics Ltd
• Heathstock Apiaries
• UMF Honey Assoc.
• Apitech
• New Zealand Honey Producers Coop

Other contributors

• NZ Manuka Ltd
• Gibbs Honeybees
• Merilyn Manley
• Harris & Megan Grainger, University of Waikato
• Jonathan Stephens, Comvita
• Steens Honey Ltd
• Airborne Honey
• Many other beekeepers and producers who share their samples and knowledge