# From Nectar to Glass: *Quantification of Methyl Glyoxal in NZ Mānuka Honey and Mead*

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## Mead

Mead is an **ancient alcoholic beverage** made from fermented **honey and water**. Recently, mead has come back from the brink of obscurity and seen a global resurgence in popularity.

## Mānuka honey

Mānuka honey contains the potent bioactive compound **methyl glyoxal (MGO).** MGO has significant and well-established **antibacterial** properties,[1] and is responsible for mānuka honey's international fame. High MGO-honey's fetch **premium prices** in NZ and abroad.

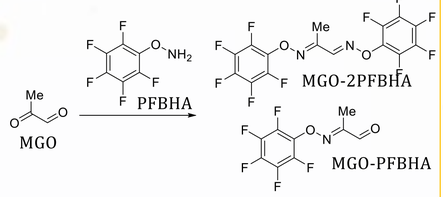
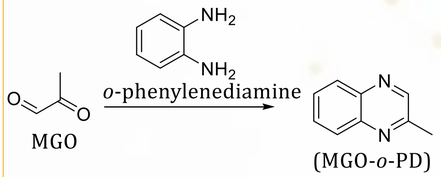
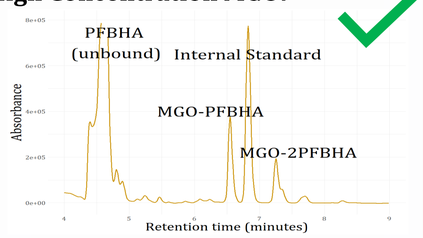
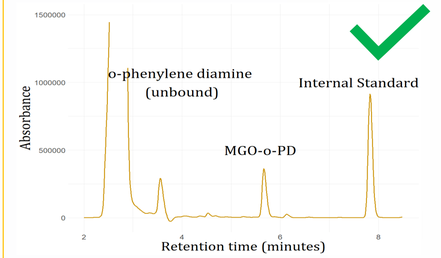
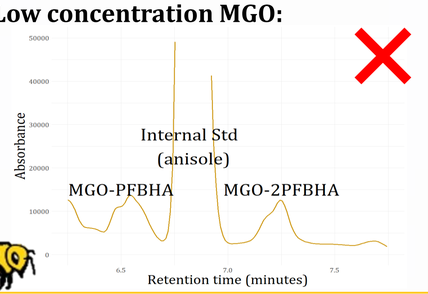
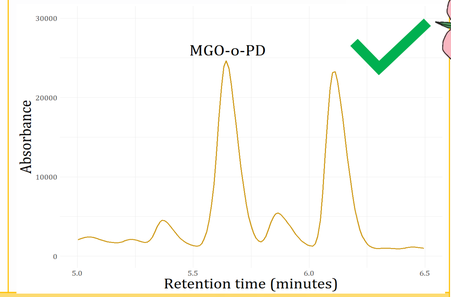
## Mānuka mead... MGO?

In previous **qualitative** studies, we've shown that **MGO is not consistently preserved through the fermentation** of mānuka honey mead – most of the time it doesn't survive the fermentation process. To further probe this mechanism, **a quantitative method** was needed for its analysis.

## Quantitative method requirements

- MGO has been quantified in mānuka honey – but **never in mead**. We set out to develop an **HPLC method.**
- MGO requires derivatisation to be visible on an HPLC instrument. **Two options for derivatising agents**, PFBHA[2] and *o*-phenylene diamine,[3] have been used


## Comparison of derivatisation methods

| PFBHA | *o*-Phenylene diamine |
|---|---|
|  |  |
| **Sample Preparation:**<br>✓ Simple sample prep<br>✓ One hour incubation period<br>✗ Some phase splitting<br>✗ Precipitate at higher MGO concentrations | ✓ Simple sample prep<br>✓ Overnight incubation period<br>✓ Homogeneous samples |
| **High Concentration MGO:** ✓<br>  | ✓<br>  |
| **Low concentration MGO:** ✗<br>  | ✓<br>  |

***o*-Phenylene-diamine method selected** due to better performance at lower MGO concentration (**greater sensitivity**), and fewer sample prep issues.

Subsequently, the method was **optimised** at all steps:
- Sample preparation
- Incaution period/derivative stability
- HPLC instrument method

Then assessed for sensitivity:
✓ LoQ = 0.58 $mgL^{-1}$ in honey, 6.35 $mgL^{-1}$ in mead
And repeatability:
✓ Experimental recovery 98-103 %, %RSD <4.5%

## Survey of commercial NZ meads

The method was applied to a survey of **all commercially available (explicitly) mānuka** (n=8), and three non-manuka meads.

| | Honey type | MGO ($mgL^{-1}$) | Sugars (g $L^{-1}$) |
|---|---|---|---|
| **MM1** | Mānuka | **4.11** | **110.3** |
| **MM2** | Mānuka | Not detected | 9.0 |
| **MM3** | Mānuka | Not detected | 0.4 |
| **MM4** | Mānuka | **7.50** | **85.6** |
| **MM5** | Mānuka | Not detected | 18.6 |
| **MM6** | Mānuka | Not detected | 49.3 |
| **MM7** | Mānuka | **19.86** | **41.7** |
| **MM8** | Mānuka | Not detected | 43.6 |
| **RM1** | Rewarewa blossom | Not detected | 41.2 |
| **KM1** | Kāmahi blossom | Not detected | 1.9 |
| **PM1** | Pōhutukawa blossom | Not detected | 9.1 |

## Findings

**Only three** of the commercial mānuka meads tested (MM1, MM4 & MM7) **contained detectable levels of MGO**.

MM7 was noted to have been ***back-sweetened*** with mānuka honey - honey was added into the finished mead **after fermentation**.

The other samples with MGO, MM1 and MM4, may also have been **back-sweetened as they have high sugar** content.

## Conclusions

- Our developed method can **efficiently quantify MGO in mānuka honeys and meads**
- **MGO** is NOT consistently present in mānuka meads –likely **does not survive fermentation**.

### Impact

Our results show that producers should choose to **reserve their more expensive mānuka honey for back-sweetening** if an MGO-containing mead is desired.

### Next steps

To further probe the interaction of yeast with MGO, we will apply the developed method to **controlled lab-scale** mānuka honey ferments – quantify MGO **pre, mid, and post fermentation**.
Influence of:
- MGO content of honey used?
- Yeast strain?
- Fermentation conditions?

**References**
1) *J. Apic. Res.* **1988**, *27* (1), 62–67. https://doi.org/10.1080/00218839.1988.11100783
2) PLoS ONE 2016, 11 (11), e0167006. https://doi.org/10.1371/journal.pone.0167006.
3) *Carbohydr. Res.* **2008**, *343* (4), 651–659. https://doi.org/10.1016/j.carres.2007.12.011.

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