Diastase; a disappearing act in Mānuka Honey

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BACKGROUND

- New Zealand's most exported honey by volume is mānuka and therefore it has a significant contribution to NZ's multi-million dollar apiculture industry.[1]

- Unfortunately, high grade mānuka honey often **fails the diastase test** due to rigorous testing and because...
  1. Mānuka honey contains **naturally low diastase activity**.[2]
  2. Diastase activity **decreases more rapidly** in mānuka honey than in non-mānuka honey.[2]

- **Diastase test** = measures the activity of the enzyme diastase.

- **Diastase** is a **56 kDa α-amylase** added to honey unintentionally by honeybees. This enzyme **catalyses the hydrolysis of the α-D-(1-4) glycosidic bond** found in starch (an α-amylose and amylopectin biopolymer).[2]

- The mānuka marker **methylglyoxal (MGO) is highly reactive** and is **known to form** advanced glycation end products (**AGEs**).[2]

HYPOTHESIS:

In mānuka honey, **MGO is forming AGEs on highly reactive amino acid residues of diastase which interfere with the function of diastase**, thus decreasing the diastase activity in mānuka honey.

AIMS

1. Measure the diastase activity and concentrations of compounds present in mānuka honey over time to observe correlations.

2. Obtain a structure of *A. mellifera* (honeybee) diastase and identify the sites at which AGEs are forming on it.

METHODOLOGY

Experiments for aim 1:	▪ Time trial (2 mānuka honeys and 6 clover honeys with added compounds) ▪ 3-in-1 MGO and DHA quantification method[2] ▪ Phenolic compound quantification method using LC-MS ▪ Pseudo-continuous colorimetric plate diastase activity assay (**adapted from the IHC Schade method)**[3]
Experiments for aim 2:	▪ Recombinant diastase expression in the Origami *E.coli* strain ▪ Purification of recombinant and native diastase expression ▪ Crystallography of recombinant diastase using robotic & crystal screens and X-ray diffraction ▪ LC-MS/MS of protein bands and purified protein

RESULTS & DISCUSSION

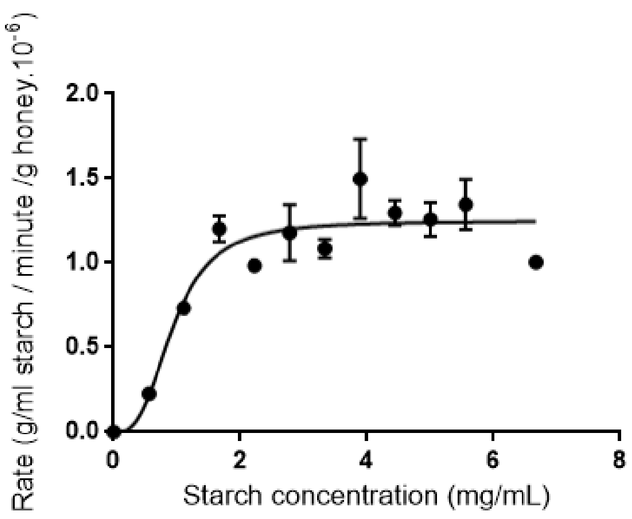

Figure 1. The allosteric-sigmoidal plot for diastase activity in fresh clover honey.

Table 1. The parameters from the allosteric-sigmoidal plot of fresh clover honey (figure 1).

Parameters	
Vmax	1.248
h	2.982
K_{half}	0.1052
R^2	0.8797

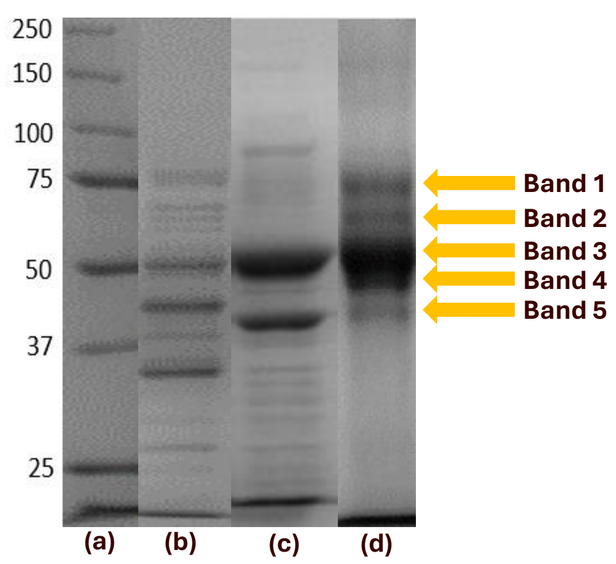

Figure 2. The size exclusion of recombinantly expressed diastase in the Origami strain of E.coli for (a)-(c) and dialysed honey (d). (a) Protein ladder (kDa). (b) First attempt. (c) Improved purification (second attempt). (d) Fresh clover honey.

Table 2. The protein identification (using LC-MS/MS) of the bands from the dialysed fresh clover honey.

Band	% coverage of α-amylase	% coverage of α-glucosidase
1	Absent	76.7
2	Absent	22.1
3	59.4	25.2
4	16.8	16.2
5	Absent	12.5

- The diastase activity assay has been developed and the method validation results are being processed.
- Diastase appears to have an allosteric-sigmoidal fit (**figure 1 and table 1**) for its activity indicating that there is more than just the active site and substrate involved in the degradation of starch.
- Recombinant diastase has been successfully purified (**figure 2)** and robotic screens have been set-up for crystallography.
- Native diastase could not be completely purified from honey as identified by **table 2**.

FUTURE WORK

- Observe sites and number of modifications occurring on diastase
- Do an inhibition assay
- Send crystals to Australia for x-ray diffraction
- Spike artificial honey with purified protein and mānuka markers.

References:
(1) *New Zealand Honey Exports*; 2023. https://www.mpi.govt.nz/dmsdocument/42360-New-Zealand-honey-exports (accessed 29 April 2024).
(2) Bell, A. R.; Grainger, M. N. C. Accelerated loss of diastase in mānuka honey: Investigation of mānuka specific compounds. *Food Chemistry* **2023**, *426*, 136614. DOI: https://doi.org/10.1016/j.foodchem.2023.136614.
(3) Commission, I. H. *Harmonised Methods of the International Honey Commission*; 2009. https://ihc-platform.net/ihcmethods2009.pdf (accessed 3 August 2024).

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