

Investigation of Temporal Apparent C4 Sugar Change in Manuka Honey

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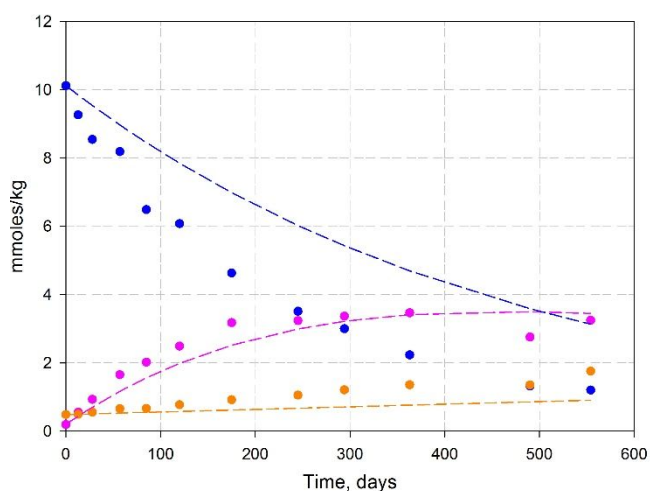
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Supplementary materials

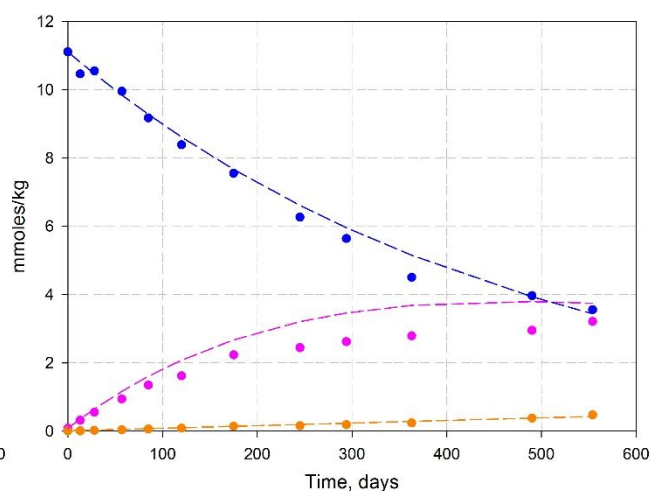
Table S1. The results of retesting a set of manuka honeys with Bruker Honey-Profiling™ NMR method (adopted from a conference presentation¹¹).

Sample #	A	B	C	D	E	F	G	H
$\delta^{13}\text{C}_h$	-24.70	-25.72	-25.16	-24.82	-25.62	-25.36	-25.51	-25.45
$\delta^{13}\text{C}_p$	-27.06	-27.61	-26.91	-26.18	-26.61	-26.03	-27.24	-26.10
Apparent C4 sugar %	13.6	10.6	10.2	8.3	5.9	4.1	9.9	4.0
DHA, mmol/kg	4.9	4.2	5.7	20.0	23.1	30.1	5.0	28.0
MG, mmol/kg	8.5	10.6	13.0	5.5	5.0	6.1	11.8	6.2
HMF, mmol/kg	0.9	0.6	0.7	0.1	0.1	0.1	0.7	0.1
Bruker Honey-Profiling™ NMR test results								
Sugar syrups	No	No	Yes	No	No	No	No	No

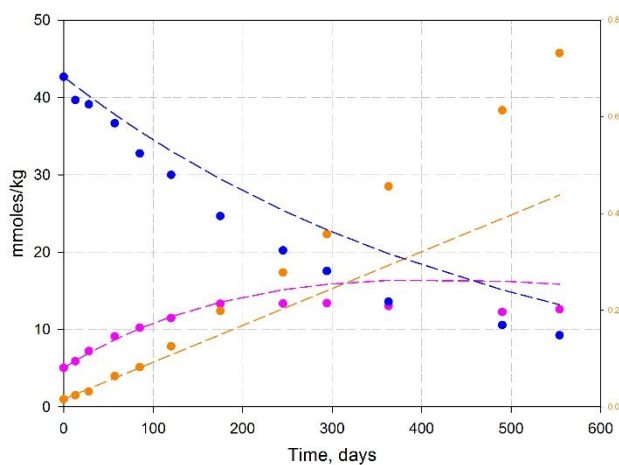
01_NIUE_DHA



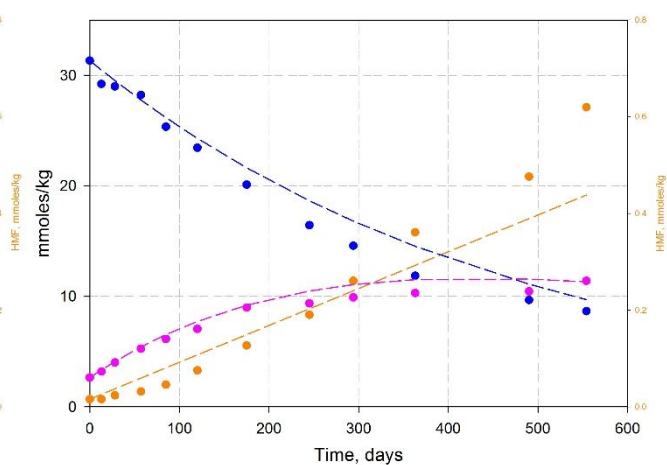
02_CLOVER_DHA



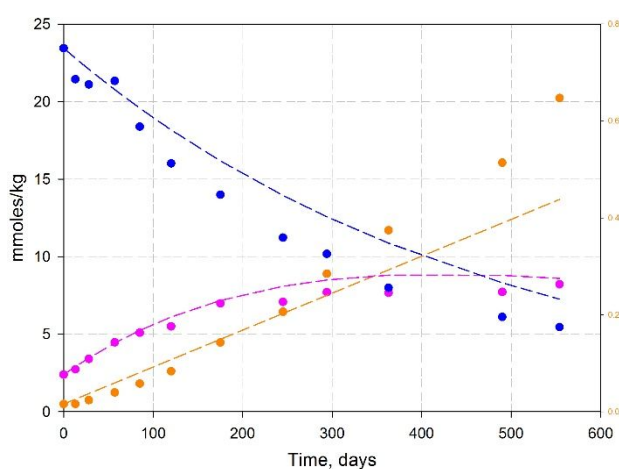
04_MANUKA



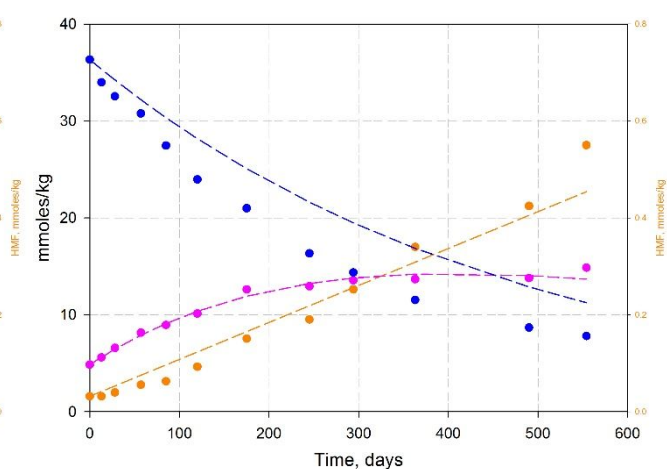
05_MANUKA



06_MANUKA



07_MANUKA



08_MANUKA_OLD

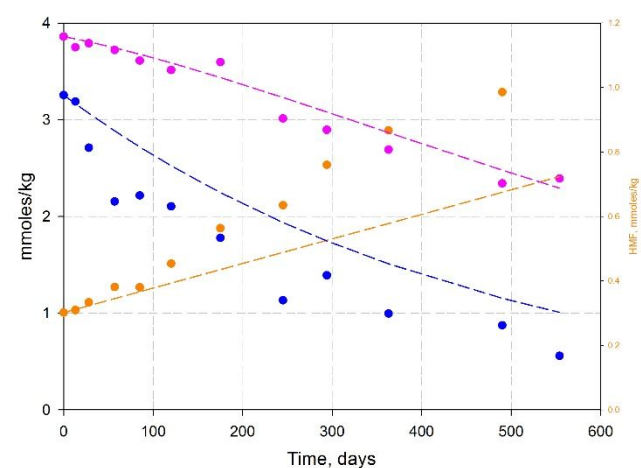


Figure S1. Change in concentrations of DHA (blue), MG (magenta), HMF (brown) over time in the studied honey samples. Dots — experimental values; dashed lines — forecast by Analytica's kinetic model. Sample #3 (pure clover honey) is not shown.

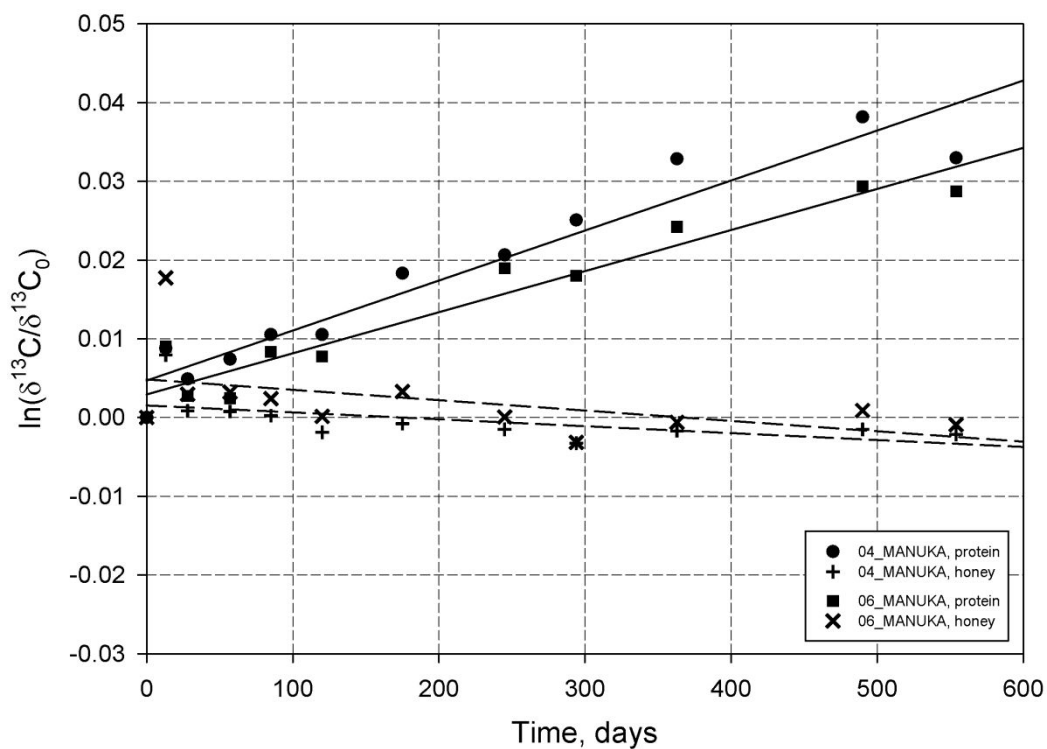


Figure S2. The plots of $\ln(\delta^{13}\text{C}/\delta^{13}\text{C}_0)$ vs. time for the other two high quality manuka honeys (group 3). The grey dashes are 95% confidence intervals.

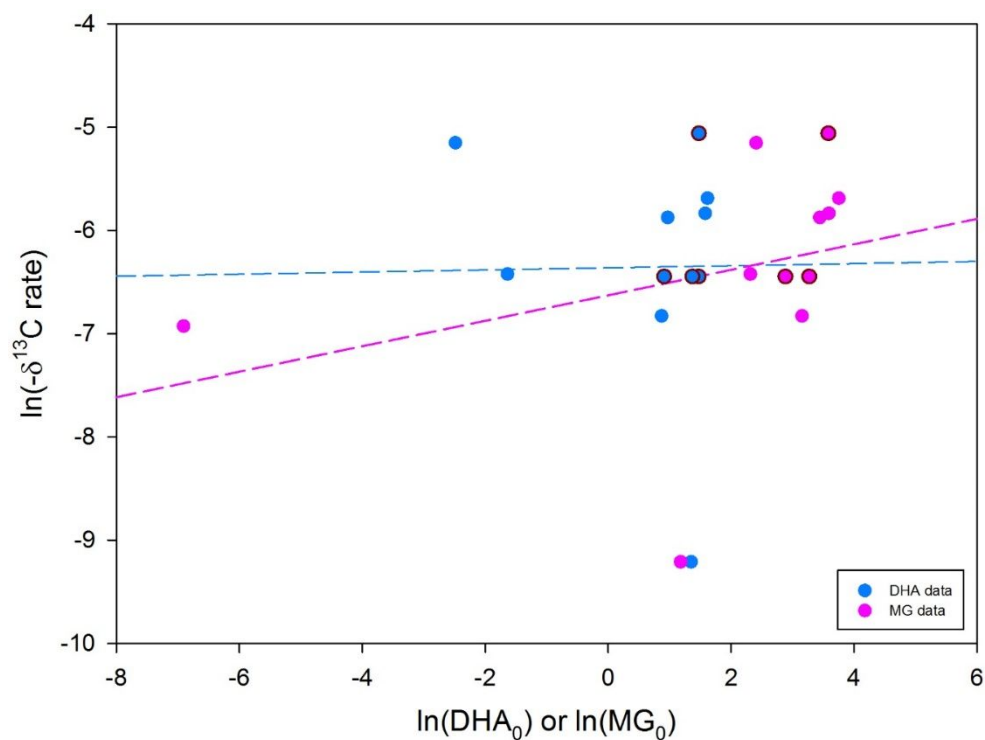
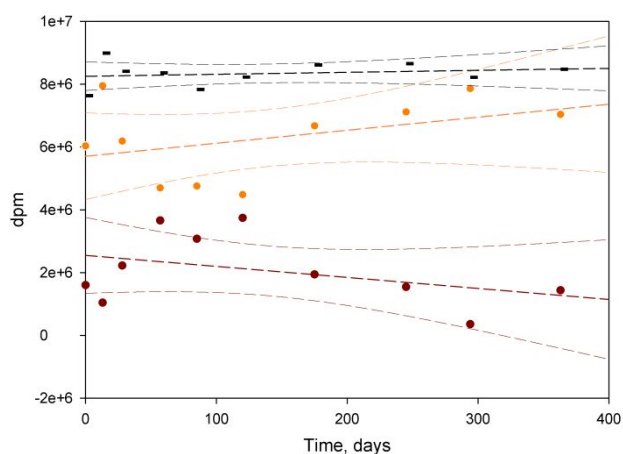
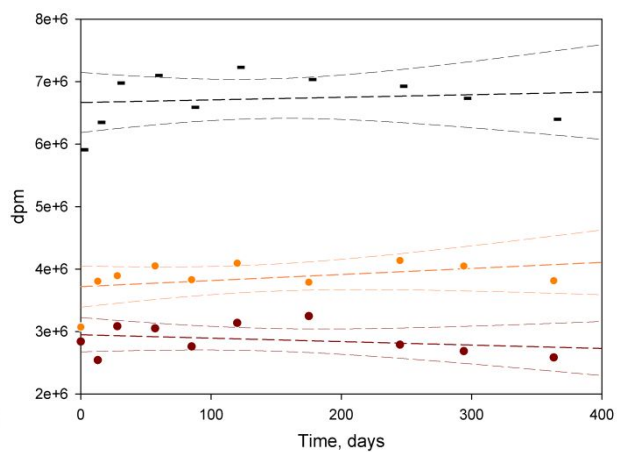


Figure S3. The plot of $\ln(-d\delta^{13}\text{C}/dt)$ vs. $\ln(\text{DHA}_0)$ and $\ln(\text{MG}_0)$. The plot includes the data of Rogers¹ (bordered circles).

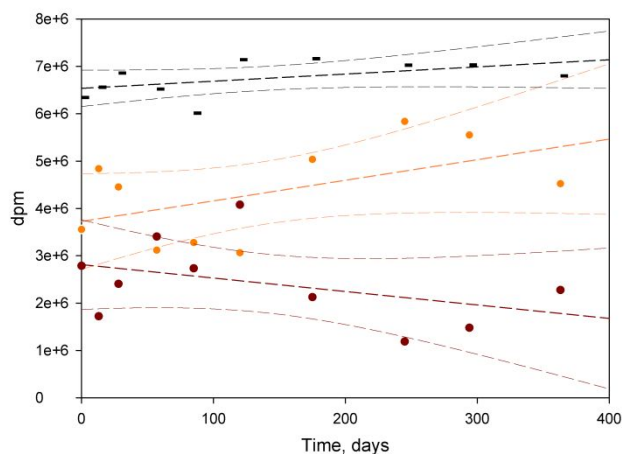
01_NIUE_DHA



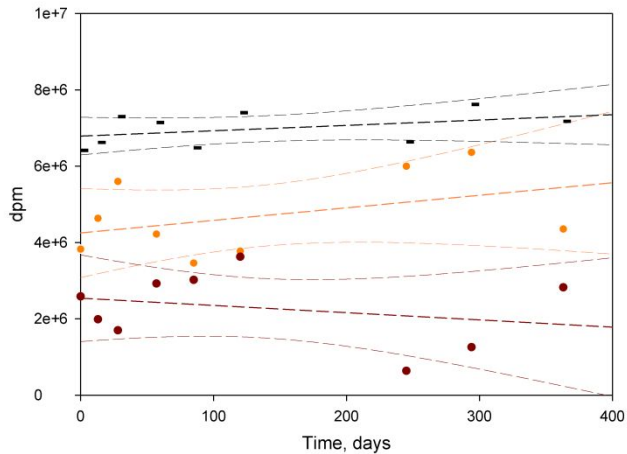
02_CLOVER_DHA



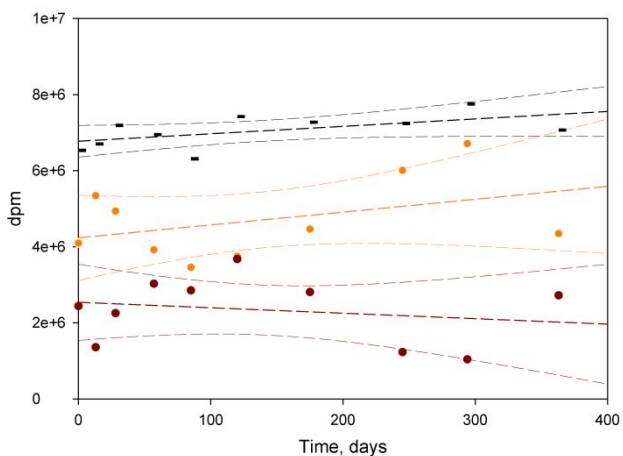
04_MANUKA



06_MANUKA



07_MANUKA



08_MANUKA_OLD

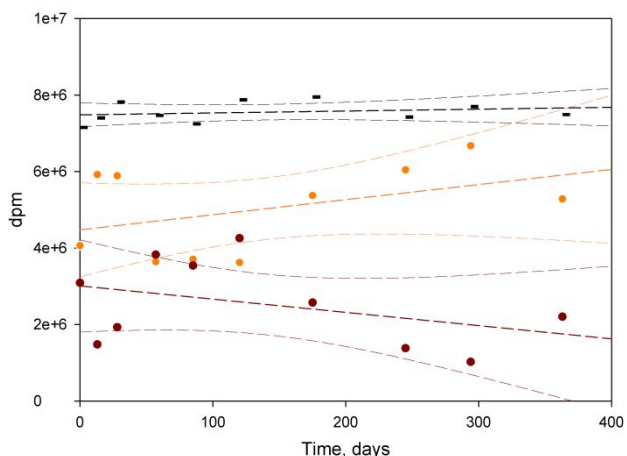


Figure S4. Change in scintillation counts, with 95% confidence intervals, for honey (light brown), protein (dark brown), and total (black) over time in the studied honey samples. The black dashes show the total amount of ^{14}C label in the sample, and serve as a control to check in there was any loss of the labelled material during the protein precipitation.

Table S2. The results of isotopic headspace analysis of the honey samples.

Sample ID	O ₂ , %	CO ₂ , ppm	δ ¹³ C
Laboratory air	20.5	770	-23.4
01_NIUE_DHA	20.3	1260	-27.6
02_CLOVER_DHA	19.2	1500	-29.8
03_CLOVER	19.3	6530	-26.4
04_MANUKA	18.8	12110	-35.2
05_MANUKA	18.4	4840	-35.0
06_MANUKA	18.0	12300	-37.8
07_MANUKA	19.3	1200	-25.7
08_MANUKA_OLD	19.2	6240	-34.9
Fresh, non-incubated manuka honey*	20.3	1540	-27.1

Table S3. The results of δ¹³C measurements in artificial honey. Highlighted bold is the component changing its isotopic composition.

Time	Sample	δ ¹³ C	σ(δ ¹³ C)
Day 0	Artificial Honey + DHA	-12.28	0.02
	Artificial Protein + DHA	-10.00	0.02
	Artificial Honey - DHA	-12.17	0.07
	Artificial Protein - DHA	-9.99	0.03
Day 7	Artificial Honey + DHA	-12.32	0.04
	Artificial Protein + DHA	-10.71	0.02
	Artificial Honey - DHA	-12.28	0.05
	Artificial Protein - DHA	-10.12	0.08