Misinterpretation of LC-IRMS Honey Analysis

The effect of $\delta^{13}C$ isotope fractionation of sucrose in honey by natural invertase activity

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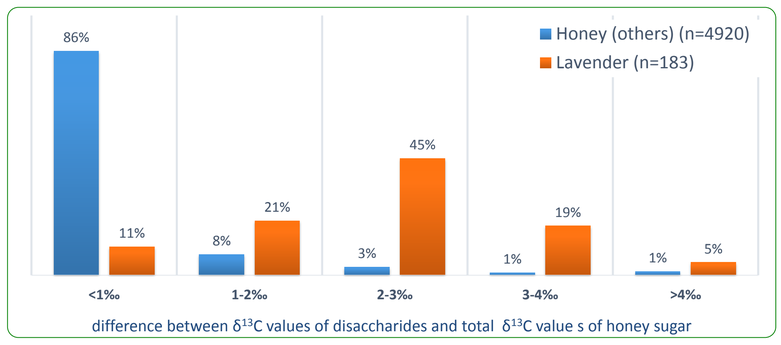
Honey is a product made of nectar which mainly consists of fructose, glucose and sucrose. Some botanical origins have typically higher concentrations of sucrose (like e.g. acacia or lavender honey). Bees are adding invertase (EC 3.2.1.26) during the digestion process to the honey (with an activity up to 250 U/kg).

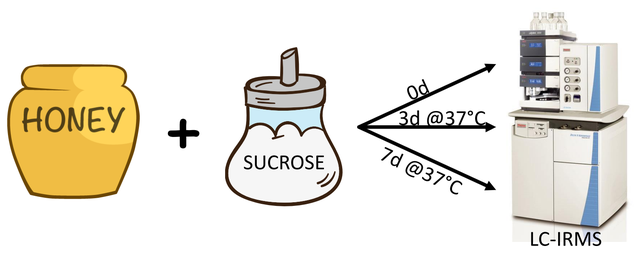
Invertase catalyzes the breakdown of sucrose into fructose and glucose.

The detection of honey adulteration with different types of starch based sugar syrups is realized by several different methods but still mainly by isotope analysis. Beside of the AOAC method 998.12 (comparison of the total $\delta^{13}C$ value of honey vs. the $\delta^{13}C$ value of honey protein) another comprehensive method[1] is used, in which the $\delta^{13}C$ value of the sugar fractions (glucose, fructose and di-, tri- and oligosaccharides) are checked for differences.

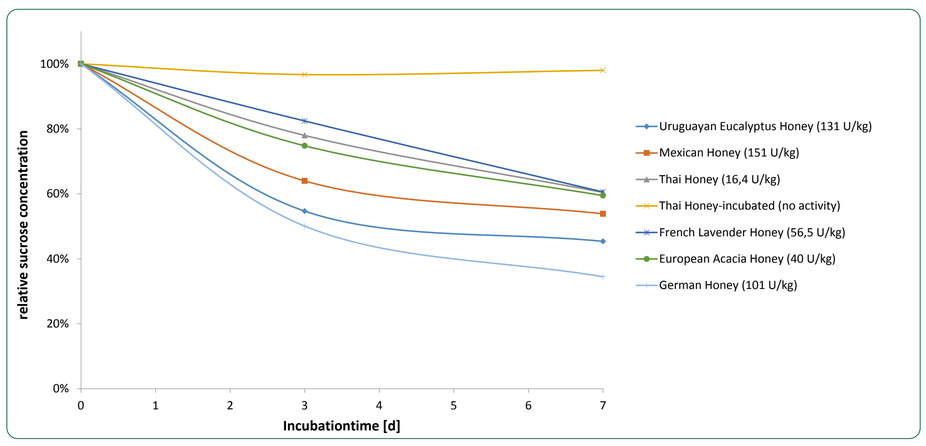
Per year QSI is analyzing more than 20,000 samples for adulteration – therefore it could be observed, that honeys with a high sucrose content indicate deviations in the $\delta^{13}C$ value of their disaccharides. In a statistical evaluation this thesis could be proven (see figure on the right) – lavender honey has a typical shift of $\delta^{13}C$ value of disaccharides vs. total $\delta^{13}C$ value of 2-3‰, whereas other honey show mostly differences <1‰.
A possible reason is the cleavage of sucrose by invertase which is linked to isotopic discrimination (as described in literature by Mauve et al.[2]) which will be investigated…

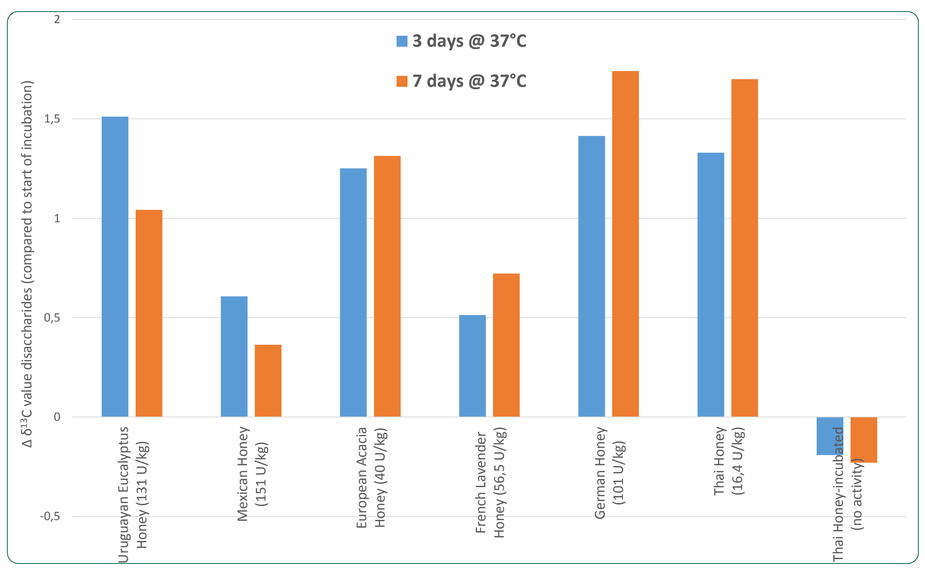


Experiment I



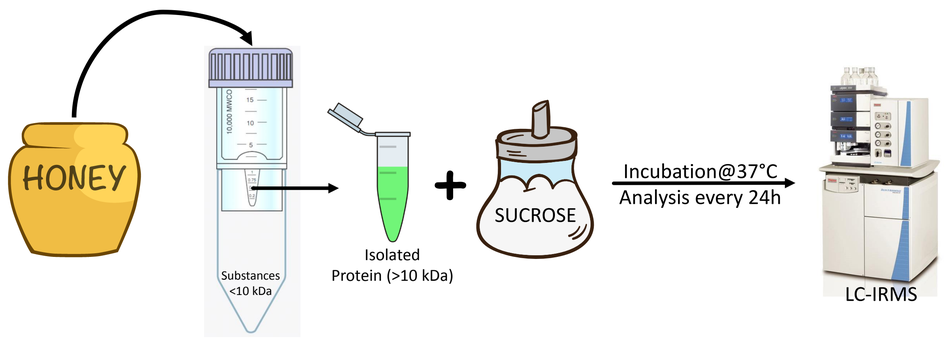
Different honeys are mixed with sucrose (+10%, $\delta^{13}C$ value: -26,67‰) and incubated at 37°C. For control a honey was incubated at 100°C untill the invertase activity was not detectable anymore.





All honeys are showing positive differences after incubation compared to the fresh honey-sucrose mixture. The isotope effect for disaccharides could be revealed also for other honeys with “normal low sucrose concentration”. To objectify the results and exclude honey side effects experiment II was performed (see right side).

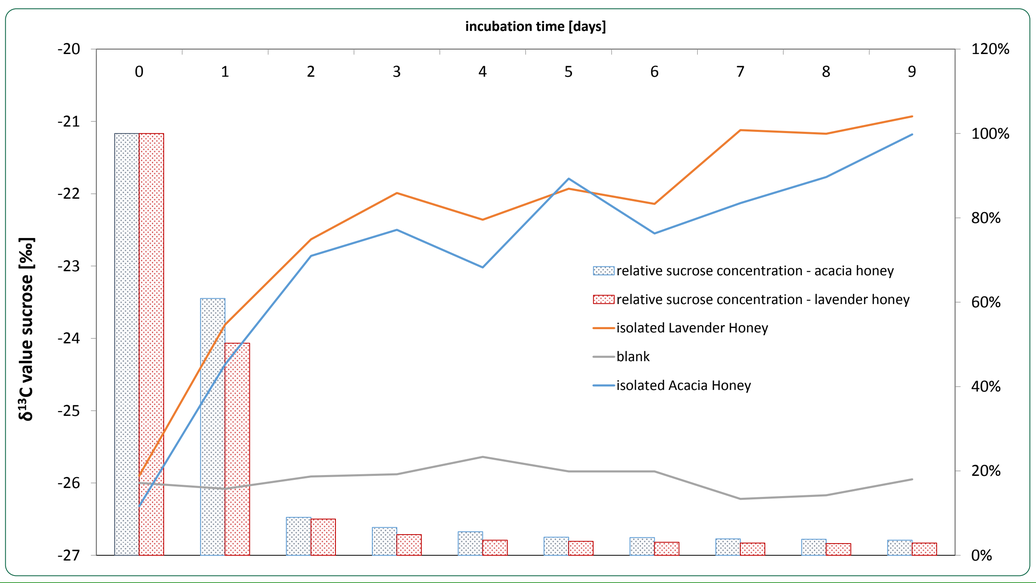
Experiment II



The protein fraction of a lavender and acacia honey were isolated by using ultrafiltration spin columns with a cut of value of 10 kDa. The cleaning success is displayed in the following table and shows good cleanup from all small molecules.

	before cleanup		after cleanup	
	Fructose conc. [g/100g honey]	Invertase activity [U/kg honey]	Fructose conc. [g/100g honey]	Invertase activity [U/kg honey]
Lavender Honey	34,9	43,1	< 0,1	47,2
Acacia Honey	37,0	26,9	< 0,1	35,1

Isolated honey enzyme was mixed with sucrose (+10%, δ13C value: -26,67‰) and incubated at 37°C. For control a honey was incubated at 100°C untill the invertase activity was not detectable anymore.



The results are showing, that the isolated honey enzymes are cleaving the sucrose (reaction is nearly in equilibrium after 3 days). Both honeys are showing the same behavior with small differences due to the enzyme activity. A strong isotope effect could be observed in accordance to the sucrose cleavage – after 3 days at the equilibrium stage the isotope effect led to a difference of nearly **4‰ $\delta^{13}C$**.

Conclusion

Both experiments are showing unequivocally, that natural honey invertase is able to discriminate the isotope values of the sugar fractions in the honey. This leads to natural differences which can be observed in honeys which are made from high sucrose nectars by the bee.

This can cause a misinterpretation as “adulterated honey”.
It should strongly be considered to not judge honeys with a natural high sucrose concentration as “adulterated”, when the disaccharide fraction in the LC-IRMS is deviating.

1: Elflein, L.; Raezke, K. P. - Apidologie 2008, 39 (5), 574-587.
2: Mauve et al. - Rapid Commun. Mass Spectrom. 2009; 23: 2499–2506

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