Positive deviations of $d^{13}C$ IRMS-values between honey and protein - effects of adulterations

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^{13}C isotope ratio mass spectrometry (^{13}C-IRMS) serves the purpose of detecting honey adulteration with foreign sugars which is established as AOAC Official Method 998.12 [1, 2]. $d^{13}C$ values of honey and precipitated protein are compared. The values should nearly be the same while negative differences indicate an addition of sugars from C4 plants.

Often sugars from C3 plants are used for honey adulterations. Predominantly syrups of beet sugar or rice starch are applied. The possible evidence of these syrups as adulteration products with AOAC method 998.12 is not much discussed yet. However, positive deviations between $d^{13}C$ values of honey and protein of more than 1.0 ‰ are noticed in many cases. The aim of this work was to proof the origin of such deviations.

Firstly $d^{13}C$ values of beet sugar (*beta vulgaris*) and rice (*oryza sativa*) of different geographical origins were determined. It is shown that these values basically were very negative [**Fig. 1**].

Consequently theoretical calculations regarding the behaviour of $d^{13}C$ values of admixtures of these sugars to honeys were carried out. These calculations revealed that, depending on the initial value of honey, the deviations of protein and honey get more positive with larger portions of foreigns sugars [**Fig. 2**].

^{13}C-IRMS measurements of natural, unadulterated honeys, which were purchased directly from beekeepers, show dispersions of $d^{13}C$ values of honey to protein in a scope of –0.9 ‰ up to +1.0 ‰ [**Fig. 3**]. These examinations are confirmed by literature [3].

Afterwards syrups of beet sugar and rice starch, respectively, were added to honeys with different grades, and these mixtures were measured by means of ^{13}C-IRMS. As a result, the findings of the calculations shown above could be confirmed: the more foreign C3 sugars the honeys contained, the more positive were the ratios of protein and honey [**Fig. 4, 5**].

But compared to $d^{13}C$ values of honey and C4 sugars, the values of C3 sugars are much closer to each other. So positive deviations mean significantly higher contents of foreign sugars than comparable negative deviations. Furthermore, it shows much higher dependency on the initial $d^{13}C$ value of honey: the higher the initial value of honey is, the less the deviation of honey and protein is affected by an admixture of C3 sugar.

Generally these examinations show that the reason for positive deviations of more than 1.0 ‰ result from an addition of C3 sugars to honey.

Revision of the AOAC Official Method 998.12 is suggested. The results of this work should be supplemented that deviations of more than +1.0 ‰ between honey and protein evidence an adulteration of honey with C3 sugars.

sample	description	geographical origin	$d^{13}C$ - average value
beet sugar	refined	Germany	-26.1
	refined	France	-26.4
	refined	Poland	-26.2
rice	*japonica*, white	China	-27.8
	japonica, whole grain	China	-27.2
	Basmati, semi-polished	India	-26.9
	Basmati, semi-polished	India	-27.4
	Hom Mali, longcorn, unpolished	Thailand	-27.4
	Lila, unpolished	Laos	-26.4

Fig. 1: measurements of C3 plants which can be used as adulteration products for honey

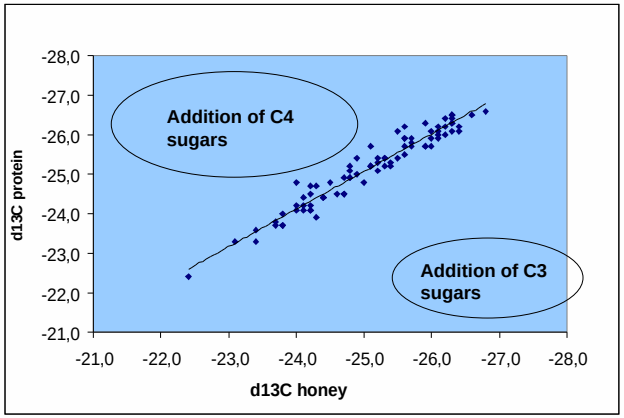

Fig. 3: dispersions of $d^{13}C$ values of honey and protein (unadulterated samples) and scopes of admixtures of C4- and C3-sugars

	initial $d^{13}C$ values of honey	admixture of beet sugar syrup $d^{13}C$ = -26.4 ‰			admixture of rice syrup $d^{13}C$ = -27.8 ‰		
		50 %	25 %	10 %	50 %	25 %	10 %
honey (h)	-24.1	-25.3	-24,6	-24,3	-26.0	-25.0	-24.4
protein (p)	-24.1	-24.1	-24.1	-24.1	-24.1	-24.1	-24.1
difference p-h	+/- 0	+1.2	+0,5	+0,2	+1.9	+0.9	+0.3
theoretical	+/- 0	+1.2	+0,6	+0,2	+1.9	+0.9	+0.4

Fig. 4: admixtures of beet syrup ans rice syrup, respectively, to acacia honey

	initial $d^{13}C$ values of honey	admixture of beet sugar syrup $d^{13}C$ = -26.4 ‰			admixture of rice syrup $d^{13}C$ = -27.8 ‰		
		50 %	25 %	10 %	50 %	25 %	10 %
honey (h)	-22.8	-24.6	-23.8	-23.3	-25.3	-24.2	-23.3
protein (p)	-22.6	-22.6	-22.6	-22.6	-22.6	-22.6	-22.6
difference p-h	-0.2	+2.0	+1.2	+0.7	+2.7	+1.6	+0.7
theoretical	-0.2	+2.0	+1.1	+0.7	+2.7	+1.5	+0.7

Fig. 5: admixtures of beet syrup ans rice syrup, respectively, to polyflora honey

initial $d^{13}C$ values of honey	admixture of beet sugar $d^{13}C$ = -26.4 ‰			admixture of rice syrup $d^{13}C$ = -27.1 ‰			admixture of rice syrup $d^{13}C$ = -27.8 ‰		
	50 %	25 %	10 %	50 %	25 %	10 %	50 %	25 %	10 %
-21.0	+2.70	+1.35	+0.54	+3.05	+1.53	+0.61	+3.40	+1.70	+0.68
-22.0	+2.20	+1.10	+0.44	+2.55	+1.28	+0.51	+2.90	+1.45	+0.58
-23.0	+1.70	+0.85	+0.34	+2.05	+1.03	+0.41	+2.40	+1.20	+0.48
-24.0	+1.20	+0.60	+0.24	+1.55	+0.78	+0.31	+1.90	+0.95	+0.38
-25.0	+0.70	+0.35	+0.14	+1.05	+0.53	+0.21	+1.40	+0.70	+0.28
-26.0	+0.20	+0.10	+0.04	+0.55	+0.28	+0.11	+0.90	+0.45	+0.18
-27.0	-0.30	-0.15	-0.06	+0.05	+0.03	+0.01	+0.40	+0.20	+0.08

Fig. 2: theoretical calculations of admixtures of beet sugar and rice syrup to honeys with different initial $d^{13}C$ values (assumption: $d^{13}C$ values of honey and protein are the same)

Conclusions

1. **Positive deviations of $d^{13}C$ values between honey and protein of more than 1.0 ‰ mean admixtures of C3 sugars ✍ AOAC Off. Method 998.12 should be revised**

2. **The determination of the amount of sugar addition is not possible because of a high dependency of positive deviations on the initial values of honeys.**

References
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[3] Rossmann, A., Luellmann, C., Schmidt, H. L., Massen-spektrometrische Kohlenstoff- und Wasserstoff-Isotopen-Verhaeltnis-messung zur Authentizitaetspruefung bei Honigen, Z. Lebensm. Unters. Forsch. 195 (1992), 307-311