Comparison of an HPTLC method with the Reflectoquant® assay for fast HMF determination in honey

A. Hošťálková[1], I. Klingelhöfer[2] and G. Morlock[2]

[1]On leave from Charles University, Czech Republic
[2]Justus Liebig University of Giessen, Germany

JLU Study life Explore the world

Introduction

5-Hydroxymethylfurfural (HMF) is a widespread process contaminant in carbohydrate-rich food and beverages. A high content of HMF in honey serves as indicator for adulteration, inappropriate or prolonged storage conditions. Nowadays the determination of HMF in honey is performed using HPLC methods or spectrophotometric methods after White or Winkler. However, reliable fast alternatives to the methods mentioned are still of high interest. Hence, a recently developed high-performance thin-layer chromatographic (HPTLC) method [1] was improved to obtain reliable findings for a wider range of honey matrices and especially when increased volumes of honey samples had to be applied. Then, a critical assessment of two fast methods was performed: The Reflectoquant® HMF assay was compared with the modified HPTLC method. Both fast methods were tested on 17 honeys of different botanical and geographical origin.

Results and discussion

HPTLC method

Honey samples were extracted with a mixture of ethanol - methanol 1:1 (v/v) and diluted with water (1.667 g/mL). Samples (6.0 µL) and standard levels were applied as 7 mm x 6 mm areas on 19 tracks. A two-step development on HPTLC plates silica gel 60 was introduced: The first short development up to 2 cm with ethanol - methanol 9:1 (v/v) was essential for HMF release from the start area. Then, the separation followed using ethyl acetate (migration distance 5 cm). Detection was performed densitometrically by absorbance measurement at 290 nm. Thus, using this chromatographic system, HMF was sufficiently separated from matrix present in honey (Fig. 1).

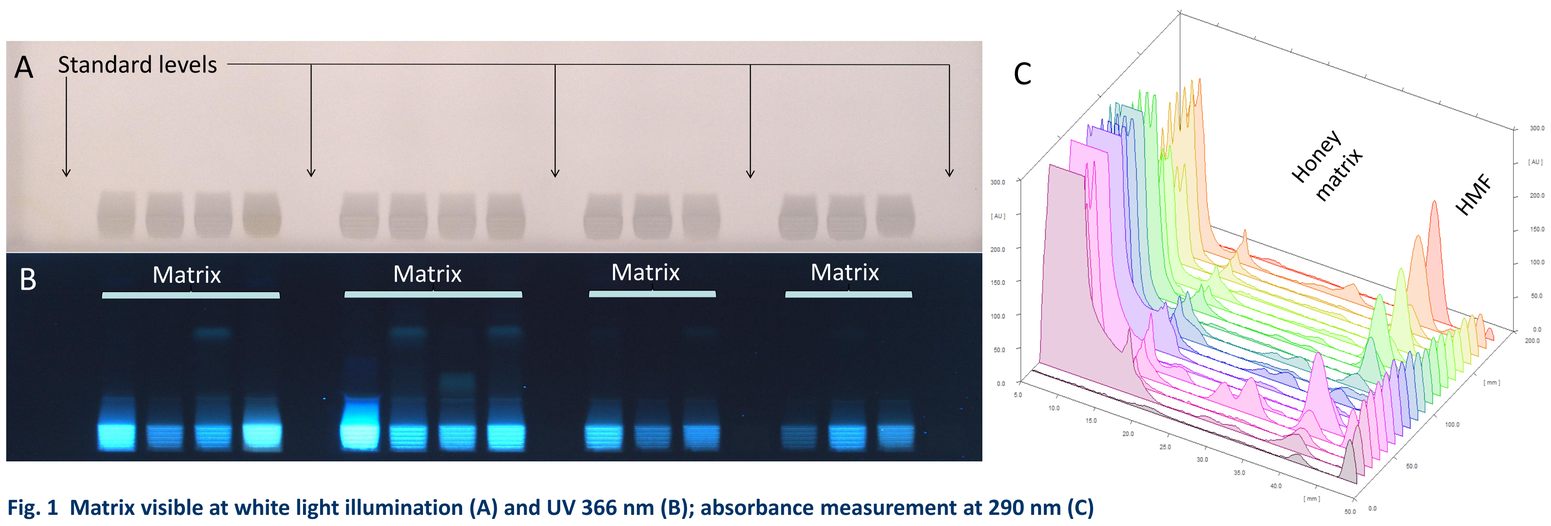

Fig. 1 Matrix visible at white light illumination (A) and UV 366 nm (B); absorbance measurement at 290 nm (C)

The reliability of the proposed HPTLC method was ascertained by evaluating various validation parameters according to ICH guidelines. HMF was satisfactorily resolved at hR_F 74 ± 1. The detection limit (LOD) of HMF was established to be < 2 ng/band (S/N 5). The quantitation limit (LOQ) was determined to be 4 ng/band (S/N 10). The analytical response in the working range (4 - 60 ng/band) showed correlation coefficients r ≥ 0.9994 (n = 8) for polynomial calibrations. The intra-day precision (repeatability, *%RSD,* n = 6*)* ranged 3.4 - 4.7 %. The inter-day precision (reproducibility, *%RSD,* n = 3) was 0.4 - 6.6 %. The reproducibility over the whole procedure inclusive sample preparation (*%RSD*, n = 2) was 0.4 - 7.2 %. Recovery rates for a range of different application volumes, and thus honey matrix applied, differed only by 4.2 %. HMF findings calculated by external calibration *versus* standard addition method (honey was spiked or oversprayed with different HMF solutions) differed on average by 2.4 % and showed that the matrix influence was minor. Hence, using this modified method the reliability and robustness of the former HPTLC method [1] was significantly improved.

Reflectoquant® assay

Aqueous honey solutions were prepared (0.25 g/mL) and the reflectometric assay was performed as specified by Merck. But for honeys with a HMF content below the LOQ of 4 mg/kg, solutions of 0.5 g/mL were prepared. For problematic chestnut colored honeys, recovery studies were performed to clarify any matrix interference.

Table 1 Method comparison of HMF concentrations in honey (mg/kg) obtained by the Reflectoquant® assay *versus* the HPTLC method

Honey sample	Mean HMF content (mg/kg) by			
	HPTLC		Reflectoquant®	
	Mean	*%RSD* (n = 2)	Mean	*%RSD* (n = 3)
Avocado Mexico	12.0	4.0	19.3[b]	6.4
Pampas Argentina	18.1	6.8	13.0	1.5
Eukalyptus Argentina	9.2	5.8	12.2[c]	0.0
Ulmo Chile	16.1	7.0	16.7	9.0
Buchweizen Europa[Δ]	56.8	6.5	51.5[b,d]	6.8
Edelkastanien Italien	4.4[a]	0.8	6.9[c]	1.5
Calluna-Heide Frankreich	8.5	7.2	8.1[c]	13.6
Klee Neuseeland	26.3	0.4	24.5	2.9
Eichenwald Spanien[Δ]	4.1[a]	5.3	8.0[b,d]	6.3
Raps Deutschland	18.9	5.5	14.6	9.6
Heide Norwegen	7.6	3.4	5.4	18.5
Quilllaya Chile	15.2	0.5	12.4	6.5
Lavendel Frankreich	42.0	3.6	39.1	5.0
Pinien Turkei	11.5	1.5	8.4	6.4
Akazien Ungarn	14.9	1.6	12.4	19.4
Blüten-Honig	15.8	3.5	15.7	7.3
Echter Deutscher Honig	56.8	7.1	58.0	8.3
Mean HMF content	19.9		19.2	
Mean deviation (mg/kg) HPTLC *vs.* Reflectoquant®	2.9		2.9	
Mean deviation (%)	14.6		15.1	

[Δ]brownish color, [a]<LOQ , [b]recovery rate outside 80-120 %, [c]c = 0.5 g/mL, [d]further diluted

Conclusions

Comparable results were obtained with both methods (Table 1) having in mind that spectrometric methods (sum parameter generated by staining) can vary up to 20 % if compared to chromatographic methods (additional separation from matrix). The mean deviation between both methods was 15 % (3 mg/kg), which underlines that both methods are well-suited for fast HMF determinations. However, precision values of the HPTLC method were superior to that of the reflectometric assay. The proposed HPTLC method simultaneously performed up to 19 analyses (2.5 min/sample) and provided a cost-effective tool for routine HMF analysis. But also the Reflectoquant® assay allowed a fast screening of honey samples (2 min/sample) excluding problematic chestnut colored honey samples which required evaluation by the standard addition method. For both methods, quantitation limits corresponded to 4 mg/kg. Hence, both methods were suited for fast quantitation of HMF in honey at the strictest regulated level of 15 mg/kg, although the modified HPTLC method seemed to be more appropriate for reliable HMF determinations in honey, as colored compounds did not interfere and the precision of the method was superior.

Acknowledgement: The project was partially co-financed by the European Social Fund and the state budget of the Czech Republic, TEAB, project no. CZ.1.07/2.3.00/20.0235 and UK SVV 267 002. Thanks to Dr. Kathrin Breitruck and Dr. Mehmet Dogan, both Merck Millipore, Darmstadt, Germany, for support with Reflectoquant® assay and plates, respectively.

Reference: [1] E.S. Chernetsova, G.E. Morlock *Anal Bioanal Chem* 401 (2011) 325-332.

IFZ Interdisziplinäres Forschungszentrum Giessen Research Centre for BioSystems, Land Use and Nutrition

Food Science, Justus Liebig University of Gießen, Institute of Nutritional Science, Heinrich-Buff-Ring 26, 35392 Giessen, Germany