Stability of Hydroymethylfurfural during Determination by HPLC

Andrè Känzig, .Daniel Kaufmann, Canton Laboratory Aarau, andre.kaenzig@ag.ch
and Stefan Bogdanov, Swiss Bee Research Centre, stefan.bogdanov@fam.admin.ch

Introduction

Hydroxymethylfurfural (HMF) is not a natural honey component and is formed by condensation of fructose upon storage and heating of honey. It is used as an indicator of honey freshness and heat damage. In Switzerland (1) and also in the EU (2), honey having more than 40 mg/kg is considered to be overheated and is not allowed to be marketed. It can only be used for industrial and bakery purposes.
Three HMF determination methods are described In the Swiss Food Manual. The old method after Winkler should not be used because of the cancerogenity of p-toluidine. The alternative measurements by HPLC (3,4,5) and by UV spectroscopy (4,5,6) will also be accepted in the new EU (7) and Codex Alimentarius (8) honey standards. These methods yielded comparable values in a ring trial, carried out with these two methods (4). For the HPLC method honey is simple dissolved in water, and after Millipore filtration HMF is determined on a reversed phase HPLC column by isocratic elution with aqueous methanol as mobile phase. It is fast as it can be automated by using an autosampler. Because of this it is the most widely used method for routine control.
There are no studies concerning the stability of HMF during the determination procedure. In the original HPLC study no time limit for the storage of the honey solutions before measurement was specified (3). The reason might lie in the fact, that at that time no autosamplers were used and the HMF content was mostly used within several hours after honey dissolution. Today autosamplers are often used and the honey solutions might be stored there for longer periods of time before determination. The reported instability of HMF in aqueous solution was encountered by chance after repeated HPLC determination of a sample, stored in the autosampler for different periods of time.

Experimental and Results

Eight market honeys were selected from the routine control samples during the autumn of 2000. Seven commercial and one industrial honeys from different geographical and botanical origin were tested. (see table 1). The water content, expressed as g/100g honey; electrical conductivity, expressed in milli Siemens per cm (mS/cm), diastase and invertase activity, expressed in diastase and invertase numbers, were determined according to the Swiss Food Manual (1).
The HMF content was determined by HPLC and UV spectroscopy after methods 9.2 and 9.3 of Swiss Food Manual (1).

The botanical origin of the honeys was determined by measurement of the electrical conductivity. According to the proposition of the International Honey Commission honeys (9) honeys with conductivity less than 0.5 mS/cm are considered to be of blossom origin, those having 0.5 to 0.8 mS/cm are blends between blossom and honeydew honey, and those with more than 0.8 mS are honeydew honeys.
HPLC was carried out immediately after dissolving the honey solution. The measurements with the two methods were practically the same.

The different analytical data in table and HMF measurements are summarised in table 1 and 2. There was a significant and rapid decrease of the HMF content as function of time in the first four honeys. This decrease is shown graphically in fig. 1. After 12 hours of storage time there was a very large decrease of the HMF content: average 50 %, minimum 32 %, maximum 82 %. In the other four honeys the decrease of HMF for the same storage time was very small: average 6 %, minimum 3%, maximum 9 %.
The addition of Carrez stopped the break down completely. This points out, that the instability of HMF is caused by a honey constituent, which is precipitated by the Carrez solution.
There was a significant correlation between the combined enzymatic activity (diastase and invertase) and the break down after 12 hours: $r = 0.799$, $p = 0.017$. This significant correlation does not mean that the break down is catalysed by one of the honey enzymes. As these two honey

enzymes are of bee origin, this correlation might also mean, that the factor, responsible for the HMF break down has bee origin.

Conclusion

The future HMF measurements by HPLC should be carried out in a way to exclude the HMF break decomposition. For that purpose the sample preparation should be carried according to the White method for HMF determination (5). After filtration the filtrate can be analysed by HPLC.

References

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Table 1. Measurements of HMF and different other parameters in commercial honeys

Geographical Origin	botanical origin	Water g/100 g	Cond. mS/cm	D Nr	S Nr	HMF mg/kg	HMF mg/kg	Break-down
1. France	Blend blossom-honeydew	16.4	0.64	27.9	5.6	59	63	Yes
2. France	Blend blossom-honeydew	17.0	0.79	23.0	3.5	17	24	Yes
3. Switzerland (Aargau)	blossom	16.2	0.30	18.5	7.6	20	23	Yes
4. Switzerland (Aargau)	blossom	15.8	0.36	26.7	19.3	15	16	Yes
5. Switzerland (Aargau)	Blend blossom honeydew	15.4	0.61	24.1	7.9	27	23	No
6. "foreign"	blossom	17.8	0.30	13.7	2.1	58	61	No
7. Mexico	blossom	16.2	0.27	12.9	0.5	117	114	No
8. Central+South Aamerika	blossom	17.7	0.35	5.6	0.4	326	314	No

Table 2. Measurements of HMF in commercial honeys

	t= 0 HPLC mg/kg	t=12 h HPLC mg/kg	+ Carrez, t=140‘ HPLC mg/kg	White mg/kg
1	63	41	59	63
2	17	12	17	24
3	22	10	20	23
4	15	3	15	16
5	27	25	27	23
6	60	57	58	61
7	118	115	117	114
8	330	314	326	314

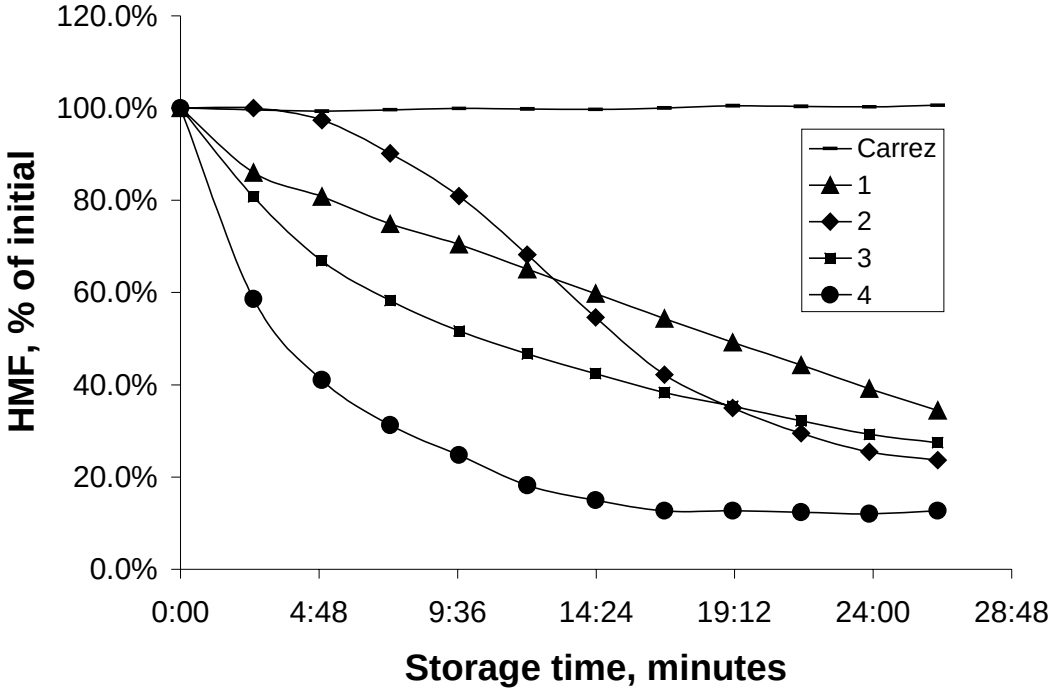

Fig. 1. Break down of HMF as a function of storage time.