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Comparison of HPLC and electrochemical determination of 5-hydroxymethylfurfural in honey and mead samples

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The content of 5-hydroxymethylfurfural (HMF) in honey and mead is strongly influenced by the technological process of these products and could be used as the parameter of choice to assess the quality of honey and mead. Therefore, the aim of this work was the comparison of well established reversed-phase high performance liquid chromatography (RP-HPLC) with voltammetric determination at the glassy carbon electrode. The final HPLC separation was performed on an Ascentis Express C18 column (150 × 3 mm, 2.7 µm) using isocratic elution with 10 % methanol in water. In total, 9 samples (6 honeys and 3 meads) were used for determination of the content of HMF.

Keywords: Honey; Mead; 5-hydroxymethylfurfural; HPLC, Voltammetry, GCE

Introduction

Honey is a natural nutritional sweetener which contains mainly carbohydrates and water. Other constituents are different compounds, such as organic acids, minerals, vitamins, pigments, enzymes, proteins, amino acids, volatile compounds, phenolic acids, and flavonoids [1]. Most of these compounds are responsible for nutritional and organoleptic properties of honey and their amount depends on many factors, such as season of production, botanical origin, geographic area, and storage conditions [2]. Mead is a traditional fermented alcoholic beverage that contains 8–18 % (v/v) ethanol and it is made from honey solutions, thus containing similar substances like the honey itself [3].

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5-hydroxymethylfurfural (HMF) is a heterocyclic organic compound derived from sugars and it is mainly formed as an intermediate in the Maillard reaction [4]. The content of 5-hydroxymethylfurfural in fresh and untreated foods is very low, but its concentration tends to increase due to heating processes or during long-term storage. Therefore, HMF is being recognized as a parameter related to the freshness and quality of the foods [5–8]. HMF is formed in honey already during maturation in honeycombs, however, only in small amount (0.6 to 2 mg kg^{-1}). An increase of HMF content is then associated with heating or storage at temperatures above 30 °C [9]. The concentration of HMF in honey is further affected by low pH values, botanical origin, aging of the honey and humidity [7]. According to the Codex Alimentarius, the limiting values of the HMF content are 40 mg kg^{-1} for common honeys and 80 mg kg^{-1} for honeys of tropical origin [10].

According to the International Honey Committee, two spectrophotometric methods and one HPLC method for the determination of HMF are recommended. Spectrophotometric variants include the determinations according to White [11] and Winkler [12], both of which are widely used in routine analysis. The principle of Winkler's approach is to measure the UV absorbance of honey solutions with the added barbituric acid and *p*-toluidine, whereas the White method involves measurements of absorbance in the UV-region in purified aqueous solutions of honey with and without bisulphite. HPLC method introduced by Jeuring and Kuppers [13] is based on a procedure, where the honey solution is separated using RP-HPLC on octadecyl silicagel stationary phase with isocratic elution using a mixture of methanol and water as the mobile phase [14]. Generally, HPLC is combined with spectrophotometric detection [15,16].

Electrochemical determination of HMF in honey can be performed by classical polarography or differential pulse polarography [17–19]. Electrode reduction of HMF may take place inside the formyl group or on the ring double bond; however, it can be assumed that the reduction in the formyl group proceeds more readily, giving rise to a specific signal in the cathodic potential range [17].

Other methods for the determination of HMF have employed micellar electrokinetic chromatography [20], anion-exchange chromatography with pulsed amperometric detection [21], or capillary electrophoresis coupled with mass spectrometry [22].

In the light of all the facts given above, this study was focused on the determination of HMF using two different techniques – well established reversed-phase liquid chromatography and direct voltammetry at the glassy carbon electrode. The differences between the results obtained using these two techniques are discussed and evaluated.

Materials and methods

Chemicals and reagents

The mead and honey samples were received from the Czech and Slovak beekeepers or bought in local markets. The standard of HMF (98%) and methanol (HPLC gradient grade) were purchased from Sigma-Aldrich (Prague, Czech Republic). Deionized water was prepared in a Milli-Q purification system (Merck-Millipore, Darmstadt, Germany). Sodium hydroxide, boric acid, acetic acid, formic acid, and phosphoric acid were purchased from Penta (Chrudim, Czech Republic) and Britton-Robinson buffer supplied by Chemapol (Prague, Czech Republic).

Instrumentation, accessories, and experimental conditions

HPLC

HPLC system was composed of two LC-20ADXR binary-gradient pump, DGU-20 degasser, SIL-20ACXR autosampler, SPD-M30 DAD detector (Shimadzu; Kyoto, Japan), and an LCO 102 column thermostat (Ecom; Prague, Czech Republic). The separation of HMF was performed on Ascentis Express C18 column (150 × 3 mm, 2.7 μm particle size) at 30 °C using isocratic elution of 10 % (v/v) methanol in water. The flow rate of the mobile phase was 0.5 mL min^{-1} and the injection volume 2 μL.

Voltammetry

All electrochemical measurements were performed on an AUTOLAB instrument (model PGSTAT-128N; Metrohm-Autolab BV, Prague, Czech Republic), into which a measuring cell with the three-electrode system incorporating the working glassy carbon electrode (GCE, with a diameter of 2 mm; Metrohm), an Ag/AgCl as the reference, and a Pt-plate as the auxiliary electrode.

Sample preparation

A quantity of 5 g honey or a volume of 5 mL mead was transferred into a 25 mL volumetric flask and filled up to the mark with water. Before the HPLC analysis, the sample was appropriately diluted by water, filtered through 0.45 μm PTFE syringe filters and analysed. For electrochemical determination, 6 mL of sample was pipetted into a voltammetric vessel and made up to the total volume of 20 mL with Britton-Robinson buffer (pH 10.86).

Similarly, different volumes of mead (according to the HMF content) were transferred into a voltammetric vessel and made up to the desired volume of 20 mL with B-R buffer solution (pH 10.86). The individual samples were measured three times (n = 3) in the case of both methods.

Results and discussion

Optimization of HPLC separation

The respective method was optimized using the standard of HMF, when the conditions and parameters of choice for separation of HMF from other compounds comprised: Ascentis Express C18 column (150 × 3 mm, 2.7 μm)**,** isocratic elution with 10 % methanol in water. The wavelength of the spectrophotometric detector was set at 280 nm. In test experiment, the retention time of HMF was found to be 5.2 min (Fig. 1).

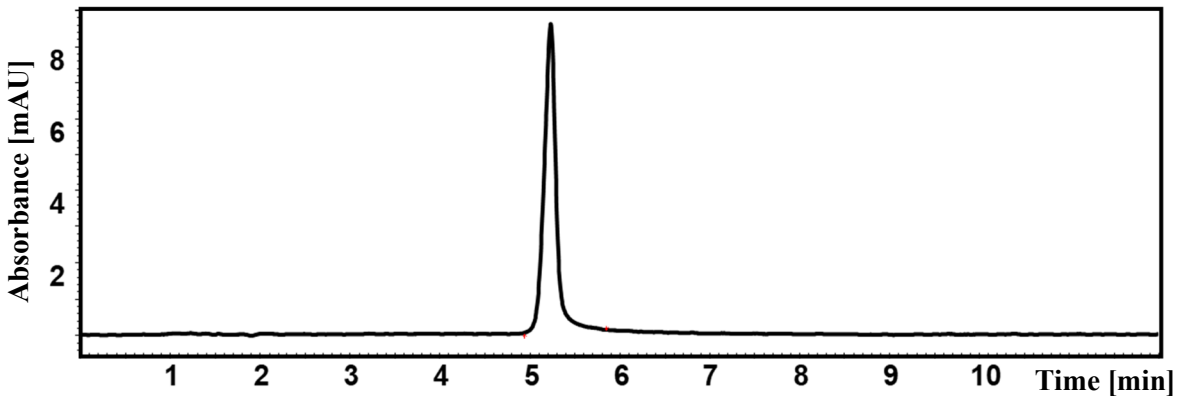

Fig. 1 Typical chromatogram after optimizing the HPLC separation
Column: Ascentis Express C18, flow rate: 0.5 mL min^{-1}, temperature: 30 °C, injection volume: 2 μL, and wavelength: 280 nm.

In the electrochemical part of the study, the HMF was determined by differential pulse voltammetry (DPV), when using the glassy carbon electrode (GCE) in Britton-Robinson buffer. The pH of buffer was optimized and the best and most stable signal of HMF was observed in a buffer solution with pH 10.86. With the increasing pH of the buffer, the peak of HMF was shifted from −1.24 V (at pH 5) to the potential of −1.51 V vs. ref. (at pH 11.76). In the buffer solution with pH 10.86, the HMF peak was detected at a potential of −1.40 V (Fig. 2).

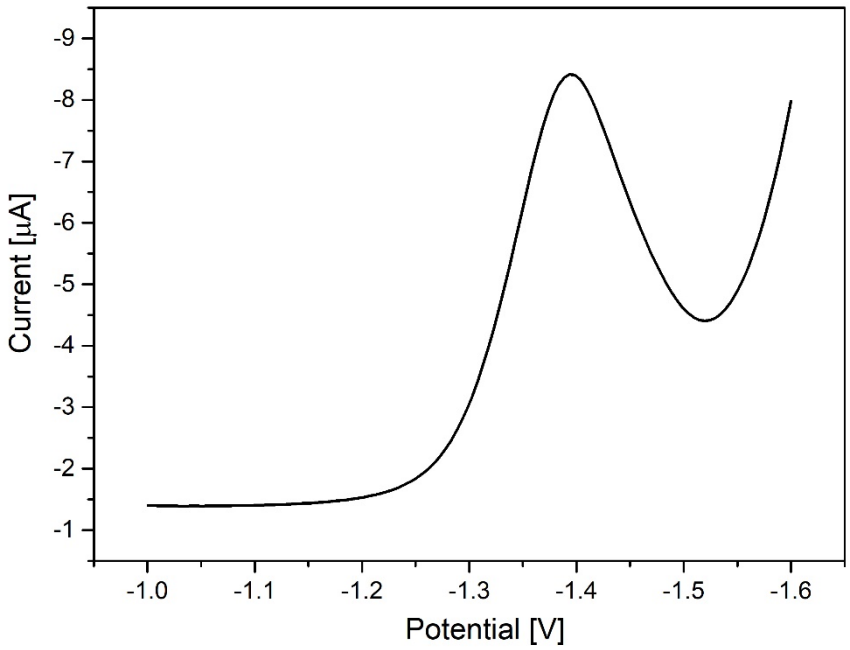

Fig. 2 Typical voltammogram for the HMF reduction

Model concentration: $5 \cdot 10^{-4}$ mol L^{-1}, working electrode: glassy carbon electrode, supporting electrolyte: Britton-Robinson buffer (pH 10.86).

Quantification of HMF

The identification of HMF was performed based on the compliance of retention times with the standards. A typical example of separation of the mead sample is shown in Fig. 3.

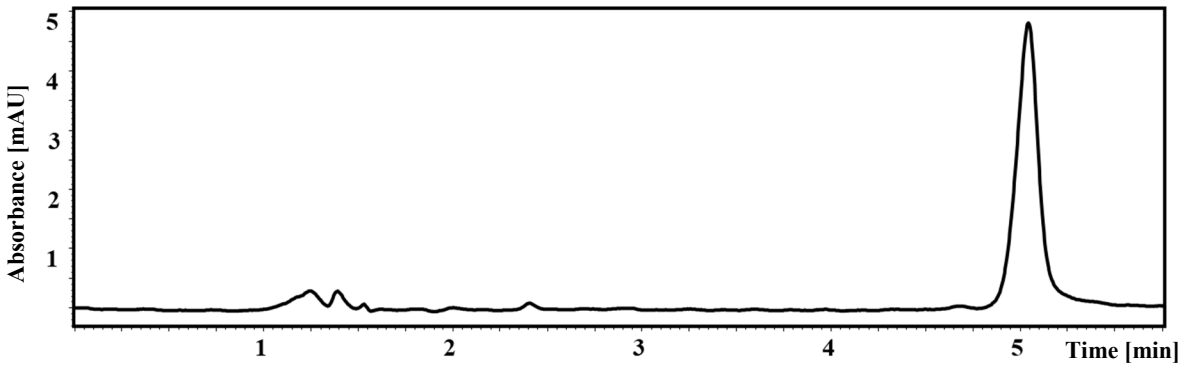

Fig. 3 HPLC analysis of mead being 15 years old

Column: Ascentis Express C18, flow rate: 0.5 mL min^{-1}, temperature: 30 °C, injection volume: 2 μL, and wavelength: 280 nm

Quantitative analysis was carried out using the calibration curve method. The concentration range of the respective solutions was 0.05–10 mg L^{-1} and the calibration data obtained at ten concentration levels, each level measured three times (n = 3).

The linearity of calibration curves was checked by inspecting the plots of residuals while the significance of intercept of regression straight-lines was tested using Student's t-test. The coefficient of determination of HMF was 0.9973, demonstrating a high linearity. Based on the regression equation (y = 1833.5 x + 0.176), the content of HMF in the samples was calculated and the results shown in Table 1.

Table 1 Comparison of the content of HMF in selected honey and mead samples measured by HPLC and with voltammetry in the DPV mode

No.	Name	Area	Concentration HPLC [mg kg^{-1}] for honey; [mg L^{-1}] for mead	Concentration DPV [mg kg^{-1}] for honey; [mg L^{-1}] for mead
1	Linden honey (warmed up) 2017	Czech Republic	7.24 ± 0.44	11.75 ± 0.61
2	Linden honey (saccharified) 2016	Czech Republic	6.83 ± 0.21	11.12 ± 0.72
3	Linden honey (fresh)	Czech Republic	1.88 ± 0.06	3.18 ± 0.06
4	Pasted honey 2017	Czech Republic	7.41± 0.10	16.83 ± 0.94
5	Pasted honey 2018	Czech Republic	0.09 ± 0.02	0.11 ± 0.03
6	Waldhonig honey	Germany	3.39 ± 0.03	4.20 ± 0.21
7	Mead from beekeeper (15 years old)	Czech Republic	215.90 ± 4.30	290.40 ± 3.90
8	Forest mead	Czech Republic	15.74 ± 0.20	17.22 ± 1.03
9	Royal almond mead	Czech Republic	165.90 ± 4.20	208.00 ± 3.40

Values given as $\bar{x} \pm s \cdot t$, where $\bar{x}$ is the arithmetic mean, s is the standard deviation, and t the critical values (2.353) of Student's t-distribution for three replicates (n = 3, α = 0.05).

The HPLC method was compared with electrochemical determination at the GCE. The quantitative analysis was performed using calibration curve method and the calibration data measured at eight concentration levels in the range 3.18–48.99 mg L^{-1}; each level three times (n = 3). The coefficient of determination was 0.9992, indicating again an excellent linearity. The regression equation for calibration data, y = 5.5617 x + 0.82538, was used for calculation of the HMF content in the samples of both honey and mead (see again Table 1).

The accuracy describing a difference between the determined and the real content of analyte was characterized by both techniques using a model sample with known concentration of 5.3 mg L^{-1} HMF. Satisfactory values of the recovery 99.8 % for HPLC and 99.3 % in the case of voltammetry were achieved.

From the results presented in the table, it is also evident, that the already established HPLC method is more suitable for the determination of HMF in mead and honey than that employing voltammetry; apparently, due to the complex matrix which had negatively influenced the voltammetric detection.

Furthermore, the results illustrate the increasing amount of HMF with the storage time. The content of HMF in fresh honey/mead samples is small, but due to the aging — and, also, warming — the samples, the amount of HMF has increased. This is also evident from the result for sample 7, where 15-years old mead has been analyzed. On the other hand, the samples 5 or 3 are fresh honeys which both had contained only small amount of HMF.

Conclusions

The content of 5-hydroxymethylfurfural (HMF) in different honey and mead samples analysed by HPLC and with voltammetry has been compared. Both techniques did not yield the same results, when a very complex matrix could be the main reason. (Probably, some compounds in the matrix were interfering with the signal of interest.) In comparison with well-established and apparently more reliable HPLC method, the voltammetric analysis has led to higher amounts of HMF found in the samples. Nevertheless, it seems that a simple voltammetry method presented herein can be used at least for orientative screening of HMF in both mead and honey.

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