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Determination of phenolic compounds and hydroxymethylfurfural in meads using high performance liquid chromatography with coulometric-array and UV detection

David Kahoun*, Soňa Řezková, Kateřina Veškrnová, Josef Královský, Michal Holčapek

Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Nám. Čs. Legií 565, Pardubice 53210, Czech Republic

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ABSTRACT

The objective of this study was the determination of 25 phenolic compounds in different mead samples (honeywines) using high performance liquid chromatography (HPLC) with coulometric-array detection and in case of hydroxymethylfurfural with UV detection. Our method was optimized in respect to both the separation selectivity of individual phenolic compounds and the maximum sensitivity with the electrochemical detection. The method development included the optimization of mobile phase composition, the pH value, conditions of the gradient elution and the flow rate using a window-diagram approach. The developed method was used for the determination of limits of detection and limits of quantitation for individual compounds. The linearity of calibration curves, accuracy and precision (intra- and inter-day) at three concentration levels (low, middle and high concentration range) were verified. Mead samples were diluted with the mobile phase at 1:1 to 1:50 ratio depending on the concentration and filtered through a PTFE filter without any other sample pre-treatment. Phenolic compounds concentration was determined in 50 real samples of meads and correlated with meads composition and hydroxymethylfurfural concentration. The most frequently occurred compounds were protocatechuic acid and vanillic acid (both of them were present in 98% samples), the least occurred compounds were (+)-catechin (10% samples) and sinapic acid (12% samples). Vanillin and ethylvanillin, which are used as artificial additives for the taste improvement, were found in 60% and 42% samples, respectively. Hydroxymethylfurfural concentration, as an indicator of honey quality, was in the range from 2.47 to 158 mg/L. Our method is applicable for the determination of 25 phenolic compounds in mead, honey and related natural samples.

1. Introduction

Traditional mead is a fermented alcoholic beverage made from bee honey and water with possible addition of spices, herbal extracts, fruit juices, etc. [1]. Studies published so far were dealt with the breeding of yeast adapted to high concentrations [2], the optimization of mead wort fermentation [3], characteristics of mead [4], monitoring of volatile compounds during honey fermentation [5], microbial flora of meads [6], the effect of heat treatment of mead wort [7] and changes in organic acid concentrations during mead wort fermentation [8].

Phenolic compounds are a widespread group of antioxidants present in plants and their derived products. Some of these compounds are taken over from plants to honey by bees (*Apis mellifera*). Phenolic compounds content in meads depends on honey and used ingredients. Phenolic compounds profile is strongly influenced by the addition of fruit juices and herbal extracts. It is also changed during technological processes, such as fermentation, heat treatment, storage, etc. These compounds affect the taste of mead (bitterness) [9–11] play a significant role in the beverages maturing [12] as they act as natural preservatives [13,14]. They also have other biological activities, such as antioxidant [15], anti-inflammatory [16], antibacterial [17] and assumed cancer-preventive effects [18,19].

Few phenolic compounds were used as the honey authenticity indicators. Discrimination of honeydew honeys and flower honeys is possible due to the difference in the concentration of protocatechuic acid [20]. Comparing of hydroxybenzoic and cinnamic acid hydroxyderivatives concentration can be used to differentiate various kinds of monofloral honeys [21]. Useful markers of heather honey could be *cis*,*trans*-abscisic acid and *trans*,*trans*-abscisic acid [22]. The major source of kaempferol and its derivatives in rosemary honey is not rosemary pollen but rosemary nectar only. These results suggest that phenolic markers of the botanical origin honey should be addressed to the identification of nectar flavonoids [23].

* Corresponding author. Tel.: +420 737916787.
E-mail address: david.kahoun@upce.cz (D. Kahoun).

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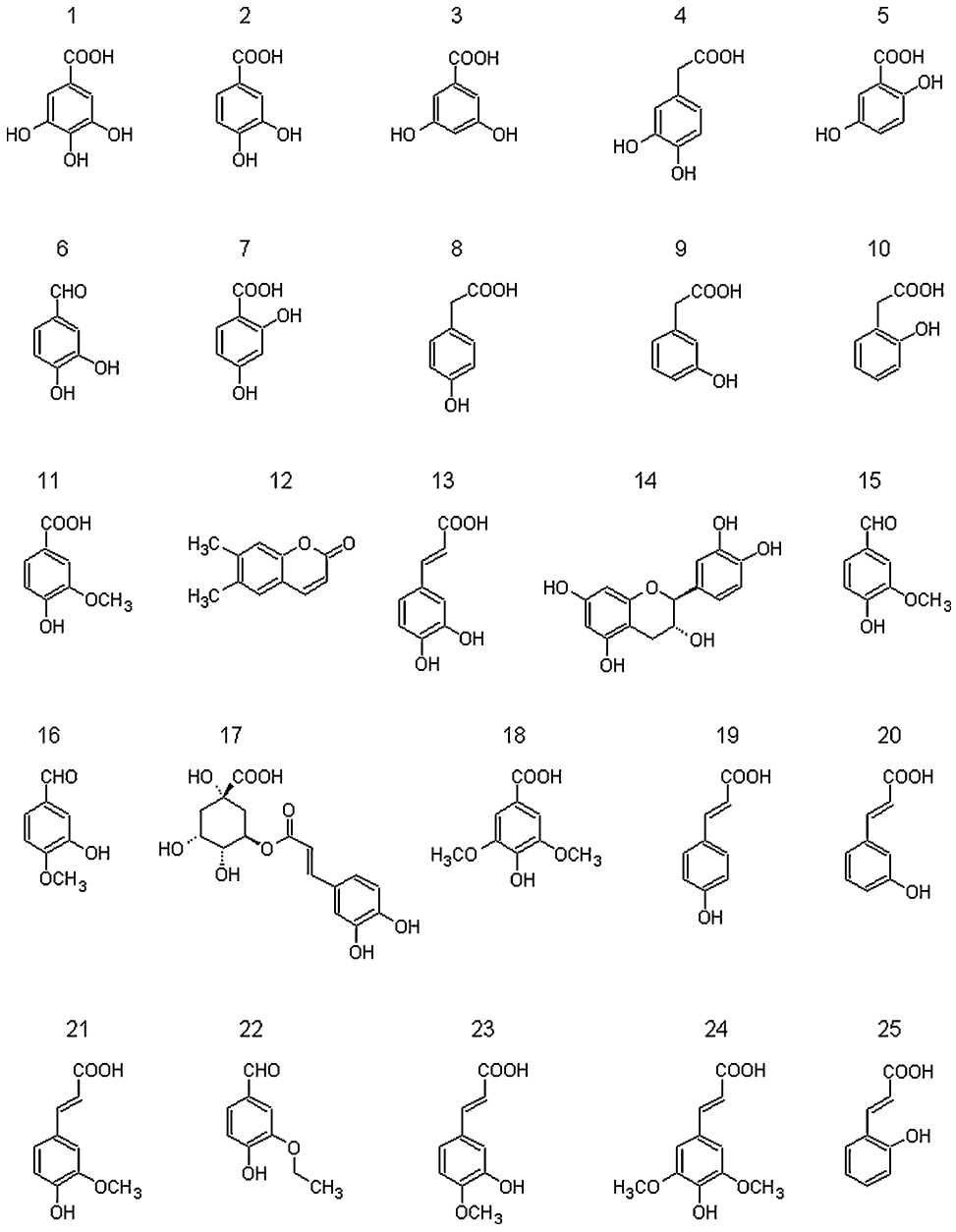

Fig. 1. Structures of the phenolic compounds.

Phenolic compounds can be useful markers for the floral origin of some honey types, particularly in heather, chestnut, eucalyptus, rapeseed and lime-tree honeys. The role of particular markers was confirmed, for example hesperetin for citrus honey, kaempferol for rosemary honey and quercetin for sunflower honey. Abscisic acid, which was indicated as a marker for heather honey, is also present in significant amounts in rapeseed, lime-tree and acacia honeys [24]. The results of comprehensive study of phenolic acids in 49 honey samples confirm significant differences of phenolic acids content depending on the floral origin [25]. It is very likely that some phenolic compounds could be used also as the indicator of mead quality and composition.

A great complexity of natural fermented beverages is a major obstacle in the determination of phenolic compounds. HPLC with coulometric-array detection is the most suitable method for analyses of these samples. This method provides a high selectivity and sensitivity, so sample pre-treatments such as extraction, purification or concentration are not necessary. This method has been successfully used for analyses of phenolic compounds in natural beverages and plant extracts [26,27], juice beverages [28], beers [29–31] and wines [32]. The determination of phenolic compounds in various beverages using HPLC method was also carried out with UV detection [33–35] or MS detection [36,37], but with a lower sensitivity in comparison to the electrochemical detection.

The objective of this study was to determine 25 phenolic compounds and hydroxymethylfurfural in 50 mead samples (honeywines) using RP-HPLC with coulometric-array and UV detection, because no systematic analytical study has been published so far on this topic. Phenolic compounds concentration was collated with meads composition and hydroxymethylfurfural concentration and dependencies were recognized. Separation conditions, such as a pH value of mobile phase, the gradient elution and the flow rate, were optimized to provide the best resolution using a window-diagram approach.