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Effect of heat treatment and storage conditions on mead composition

David Kahoun [a,*], Soňa Řezková [b], Josef Královský [b]

[a] Institute of Chemistry and Biochemistry, Faculty of Science, University of South Bohemia in České Budějovice, Branišovská 1760, 37005 České Budějovice, Czech Republic
[b] Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 53210 Pardubice, Czech Republic

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ABSTRACT

The effects of heat treatment and storage conditions on the composition of pure mead (honey wine) made from only honey and water were investigated. Heat treatment experiments were performed at 7 different temperatures ranging from 40 °C to 100 °C with 10 °C increments for 60 min. Storage condition experiments were performed at room temperature (20–25 °C) in daylight without direct sunlight and in darkness in a refrigerator at 4 °C for 1, 2, 4 and 12 weeks. The parameters evaluated were phenolic compounds, peak area of unidentified compounds, 5-hydroxymethylfurfural content and antioxidant capacity. Significant changes in compound content were observed in the case of 6 identified compounds and 9 unidentified compounds. However, the antioxidant activity was not affected by the heat treatments or storage at room temperature.

1. Introduction

Mead, or honey wine, is a fermented alcoholic beverage made from bee honey and water. The traditional production process includes the heating or boiling of honey must (unfermented solution of honey and water) before its fermentation for the purpose of protein removal and microorganism elimination (Ramalhosa, Gomes, Pereira, Dias, & Estevinho, 2011). This step reduces the risk of uncontrolled fermentation but it may also cause the degradation of thermolabile compounds e.g. some phenolic acids and flavonoids originate from honey (Escriche, Kadar, Juan-Borrás, & Domenech, 2014). Another negative consequence of these treatments is the potential increase of 5-hydroxymethylfurfural content, as is known in the case of the heat treatment of honey (Tosi, Ciappini, Ré, & Lucero, 2002). With respect to these facts, new, gentle mead production processes without heat treatment have been developed.

The chemical composition of various types of mead was evaluated in a comprehensive study of Kahoun, Řezková, Veškrnová, Královský, and Holčapek (2008) focused on the content of 25 phenolic compounds and 5-hydroxymethylfurfural in 50 meads obtained from 14 producers. The results of the study confirmed the strong effect of ingredients added to mead (fruit juices, herbal extracts, spices etc.) on their phenolic compound profile and big differences in 5-hydroxymethylfurfural content ranging from 2.74 to 157 mg/L. Similar results were also published by Švecová, Bordovská, Kalvachová, and Hájek (2015) and Beňová and Hájek (2010); in their studies they showed the benefits of HPLC with coulometric array detection for the determination of phenolic compounds in wines, meads, and Japanese knotweeds. Socha, Pająk, Fortuna, and Buksa (2015) evaluated the influence of mead type and the effect of various mead ingredients on phenolic compound profile and antioxidant activity. A principal component analysis (PCA) statistical method was used in order to differentiate meads in terms of phenolic compounds content.

It is very likely that the composition and content of phenolic compounds in meads are influenced not only by the ingredients added, but also by technological processes, such as the means of honey must preparation (Wintersteen, Andrae, & Engeseth, 2005), fermentation, maturing, storage in bottles, warming just before consumption etc. because these effects have been observed in the case of other similar fermented beverages such as wines (Fernández de Simón et al., 2014; García-Falcón, Pérez-Lamela, Martínez-Carballo, & Simal-Gándara, 2007; Matějíček, Mikeš, Klejdus, Štěrbová, & Kubáň, 2005) or beers (Vanbeneden, Gils, Delvaux, & Delvaux, 2008).

The presumption made for this research is that meads made under cold conditions contain different number of phenolic and other electroactive compounds than meads made in the traditional

* Corresponding author.
E-mail address: dkahoun@prf.jcu.cz (D. Kahoun).

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way, and that some of these compounds may be damaged by the unsuitable storage of mead in glass bottles or by its warming just before consumption (Ioannou & Ghoul, 2012). Potential changes in the chemical composition of meads may be accompanied by the formation or elimination of a compound, which could then be used as a marker of these treatments. The objective of this study was to evaluate the effects of heat treatment and storage conditions on the composition of cold-made mead using HPLC with coulometric-array detection (for electroactive compounds), HPLC with UV detection (for 5-hydroxymethylfurfural) and UV/VIS spectrophotometry (for antioxidant activity). No study has been published so far on this topic.

2. Experimental

2.1. Chemicals

Standard phenolic compounds (HPLC purity) were obtained from the 2 following sources:

Fluka (Buchs, Switzerland): gallic acid (3,4,5-trihydroxybenzoic acid), protocatechuic acid (3,4-dihydroxybenzoic acid), α-resorcylic acid (3,5-dihydroxybenzoic acid), homoprotocatechuic acid (3,4-dihydrophenylacetic acid), protocatechuicaldehyde (3,4-dihydroxybenzaldehyde), β-resorcylic acid (2,4-dihydroxybenzoic acid), 4-hydroxyphenylacetic acid, 3-hydroxyphenylacetic acid, 2-hydroxyphenylacetic acid, vanillic acid (4-hydroxy-3-methoxybenzoic acid), esculetin (6,7-dihydroxycoumarin), caffeic acid (3,4-dihydroxycinnamic acid), (+)-catechin hydrate (*trans*-3,3′,4′,5,7-pentahydroxyflavane), vanillin (4-hydroxy-3-methoxybenzaldehyde), isovanillin (3-hydroxy-4-methoxybenzaldehyde), chlorogenic acid hemihydrate (3-O-(3,4-dihydroxycinnamoyl)-D-quinic acid), *p*-coumaric acid (*trans*-4-hydroxycinnamic acid), *m*-coumaric acid (*trans*-3-hydroxycinnamic acid), ethylvanillin (3-ethoxy-4-hydroxybenzaldehyde), sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) and *o*-coumaric acid (*trans*-2-hydroxycinnamic acid).

Sigma-Aldrich (St. Louis, MO, USA): gentisic acid (2,5-dihydroxybenzoic acid), syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid), ferulic acid (*trans*-4-hydroxy-3-methoxycinnamic acid) and isoferulic acid (3-hydroxy-4-methoxycinnamic acid).

Standards of 5-hydroxymethylfurfural and Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), both HPLC purity, were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Acetonitrile and methanol, LiChrosolv gradient grade, were obtained from Merck (Darmstadt, Germany). Formic acid (98–100%), sodium acetate trihydrate (99%), acetic acid (98%), potassium dichromate (99%) and ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) tablets were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ammonium acetate (99.995%) was obtained from Fluka (Buchs, Switzerland). Water was distilled in glass and purified using a Mili-Q water purification system (Bedford, MA, USA).

2.2. Standard solutions

The standards of the phenolic compounds were dissolved in a mixture of water and methanol (1:1, v/v) to obtain 100 mg/L stock solutions and filtered through the 0.2 μm PTFE filter. The stock solutions were stored in dark brown glass vials at 4 °C in darkness for a maximum of one week. Calibration solutions ranging from 0.025 mg/L to 2.5 mg/L were prepared by diluting the stock solutions with the mobile phase A in the desired volume ratios and 10 μL of each prepared calibration solution was immediately injected to HPLC-ECD.

The standard of 5-hydroxymethylfurfural was dissolved in a mixture of water and methanol (9:1, v/v) to obtain 400 mg/L stock solution and filtered through the 0.2 μm PTFE filter. The stock solution was stored in dark brown glass vials at 4 °C in darkness for a maximum of one week. Calibration solutions ranging from 0.5 mg/L to 18 mg/L were prepared by diluting the stock solutions with aqueous methanol (9:1, v/v) in the desired volume ratios and 10 μL of each prepared calibration solution was immediately injected to HPLC-UV.

The standard of Trolox stock solution (0.004 μmol/μL) was prepared freshly before any analysis by dissolution in methanol. Calibration solutions ranging from 0.005 μmol/25 μL to 0.05 μmol/25 μL were prepared by dilution of stock solutions with the methanol in the desired volume ratios and 25 μL of each prepared calibration solution was immediately measured using a UV/Vis spectrophotometer.

2.3. Mead samples

2.3.1. Production process

The production process for pure mead, cold-made (without heating or boiling of honey must) from bee honey and water only, is the trade secret of JANKAR PROFI company, and thus can be described in general terms only. Honey (approx. 2200 kg) was gently preheated using a hot-air dryer at max. 40–42 °C and then mixed with water (approx. 3000 dm^3) in a 5000 dm^3 stainless steel fermentation tank until the appropriate value of refractometric dry solids was reached. This honey solution was inoculated with 2.5 kg of the lyophilized yeast Saccharomyces cerevisiae and fermented at 18–20 °C for 5–6 weeks until the appropriate value of refractometric dry solids was reached. During the turbulent fast fermentation, the mead was cooled to keep the temperature below 18–20 °C. Fermentation continued to completion at 16 °C for 4 weeks. The mead was then removed by suction and matured at 12–14 °C for 1 year. After maturing, primary filtration was done using a diatomaceous earth filter followed by fine filtration using a membrane filter with a 0.45 μm pore size. Finally, the mead was bottled under nitrogen atmosphere, the bottles closed with corks, and the bottle necks covered with molten wax.

2.3.2. Mead samples

The samples of fresh pure mead were obtained in two clear glass bottles from JANKAR PROFI company. Both samples (bottles) were made in the same batch. One bottle of mead was used for the evaluation of the effect of heat treatment and the other for the evaluation of the effect of storage conditions. The bottles were stored at 4 °C in darkness for a maximum of one week.

The effect of heat treatment was tested using a screw Duran Premium bottle (100 mL) with 25 mL of mead sample. The bottle was placed into a water bath at 7 different temperatures ranging from 40 °C to 100 °C with 10 °C increments for 60 min. Then, the bottle was taken out and cooled using a stream of cold water.

The effect of storage conditions was tested using an Erlenmeyer flask (250 mL) with 200 mL of mead sample at room temperature (20–25 °C) in daylight (without direct sunlight) and in darkness in a refrigerator at 4 °C for 1, 2, 4 and 12 weeks. The 12 weeks' cut-off time was chosen on the basis of the manufacturer's recommendation because this is the average period from bottling by the producer to purchase by the customer.

2.4. Instrumentation

An HPLC-ECD system for phenolic compound analysis was set up. It consisted of a vacuum degasser DG 3014 (Ecom, Prague, Czech Republic), two chromatographic pumps model 582