

Simple Method for Determination of Short-Chain Organic Acid in Mead

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Abstract Organic acids present in mead influence its fermentation rate during the production process, its stability and microbiological purity as well as organoleptic properties of the final product. The objective of this work was to validate an analytical method for determination of eight main organic acids in mead using the isotachopheresis with conductivity detection. All the validation parameters indicate high reliability of the method. The recovery rates of all analytes were between 96 and 101 %. The precision was equal or less than 2.5 %. The isotachopheretic system showed also high sensibility due to low limits of detection and quantification. For all investigated organic acids, limit of detection (LOD) ranged from 0.7 to 4.7 mg L⁻¹ and limit of quantification (LOQ) values ranged between 2.2 and 14.2 mg L⁻¹.

Keywords Organic acids · Mead · Isotachopheretic separation

Introduction

Mead is an alcoholic (9–18 %v/v of ethanol) traditional beverage, produced by fermentation of diluted (with water and/or fruit juices) honey by using different yeast strains (Pereira et al. 2009; Barata et al. 2011). Depending on dilution factor, there are different types of mead: the finest at 1:0.5 (honey/water), 1:1, 1:2 and 1:3 (Sroka and Tuszyński 2007).

The chemical composition of mead depends mostly on the composition of raw material which is specified by a floral source, geographical and environmental factors (Dezmirean

et al. 2012), added microorganisms, vinification practice which involves addition of various spices, hops and organic acids, as well as the maturation procedure (Gupta and Sharma 2009).

During the mead production process, organic acids play an important role due to their influence on fermentation rate, stability and microbiological purity, as well as organoleptic properties (e.g. flavour) of the product. There are numerous sources of organic acids in mead; however, the main ones are bee honey (the main gluconic acid source), fruit juices and organic acids (citric, tartaric or lactic acid) finally added to the bulk to ensure the optimal acidity of beverage. Moreover, some organic acids (e.g. succinic acid) are produced as by-products of ethanol fermentation.

Many analytical methods have been developed for determination of organic acids in the alcoholic beverage such as gas chromatography (Voica et al. 2013; Yang and Choong 2001) liquid chromatography techniques (Kotani et al. 2004; Kerem et al. 2004) or electrophoretic methods (Peres et al. 2009; Esteves et al. 2004). Isotachopheresis (ITP) method can be useful and cheap and an alternative tool, which allows to separate and determine short-chain organic acid in mead.

The purpose of this study was to validate the rapid and simple analytical procedure using the isotachopheresis with conductivity detection technique for determination of main organic acids present in mead.

Materials and Methods

Reagents

Deionised water of 18 MΩcm was obtained from Milli Ro & Q water purification system (Merck-Millipore, Billerica, MA, USA). Organic acids used were of analytical standard grade. Caproic acid was obtained from Fluka (Steinheim, Germany); monohydrate citric acid (Piekary Śląskie, Poland); D,L-malic

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acid and succinic acid from Lancaster (Morecambe, England); 95 % formic acid and D-gluconic acid sodium salt from Sigma-Aldrich (France) and L-tartaric acid and 80 % D,L-lactic acid from Avantor Performance Materials Poland S.A. (Gliwice, Poland). A 36–38 % hydrochloric acid was from Baker Analyzed and methylhydroxyethylcellulose (M-HEC) from HERCULES (Prague, Czech Republic), β -alanine from Merck (Darmstadt, Germany) and L-histidine was from Serva (Heidelberg, Germany); 99.5 % acetic acid was obtained from Chempur (Piekary Śląskie, Poland).

Apparatus

At the beginning of the present study, the previously reported method of determination of organic acids was modified and adopted to the analysis of mead (Dobrowolska-Iwanek et al. 2014). The previously elaborated method of determination of organic acids in wine could not be directly applied because not all acids found in mead could be separated (e.g. at pH=3.5 analytical signals from formic and tartaric acids overlap). Isotachophoretic separations were performed using the Electrophoretic Analyser EA 202 M (Villa Labeco, Spisska Nova Ves, Slovakia) with the conductivity detector. The system was equipped with sample valve of 30 μ L fixed volume and two capillary: the pre-separation capillary (90 mm \times 0.9 mm I.D.) and the analytical capillary (160 mm \times 0.3 mm I.D.). The pre-separation involved the current of 250 μ A, while the actual separation in the analytical capillary column was performed at 60 μ A during the initialization phase and at 50 μ A during the detection phase. The leading electrolyte was 15 mM hydrochloric acid with 0.2 % M-HEC, finally adjusted to pH 3.6 with β -alanine. The terminating electrolyte contained 5 mM caproic acid and 5 mM L-histidine.

Samples and Sample Preparation

The samples were purchased from the main mead producer in Poland—“Pasięka Marcin Jaros” (Tomaszów Mazowiecki,

Poland). Two types of mead were investigated, including the following: 1:1 and 1:2 honey-to-water wort (the most frequently purchased in Poland). The samples were diluted 1:20 with deionised water, as the preliminary experiments had shown that in case of non-diluted samples, the concentration of investigated organic acids exceeds the working range of the instrument. The obtained solution of each sample was injected directly into the ITP analyser without any other sample treatment.

Method Validation

Validation was performed by evaluation of such parameters of the method as: linearity range, limits of detections and quantification, precision and accuracy calculated as the recovery. The acquired zones in the ITP analysis were identified by comparison with step heights of standard solution containing pure compounds (organic acids) and of spiked samples to ensure correctness of identification. For quantification of organic acids, the external calibration curve was calculated by the analysis of calibrators at following concentration levels: 3.125, 6.25, 12.5, 25, 50 and 100 mg L⁻¹ for tartaric, formic, citric, malic, lactic, succinic, acetic and gluconic acids. The linearity range was assessed by plotting zones length corresponding to each analyte against acids concentration and then applying the least squares method for calculation respective correlation coefficients (R). According to the validation protocol, the determination coefficient had to meet the criterion of $R^2 > 0.995$. The precision of the method (expressed by the RSD value) was established by successive analysis of the non-spiked and spiked sample solutions, six independent repetitions for each, at the same working day. The validation criterion for precision was ± 2.5 % RSD. The recovery was determined by spiking the sample with known concentrations of analytes at two concentration levels (lower +25 % amount of analyte, higher +50 % of analyte amount determined in previous measurement of the same non-spiked samples). The sample prepared in the way described above was

Fig. 1 Isotachophoretic separation of organic acids in a standard mixture of eight acids (isotachophoregram **a**) and in mead sample (isotachophoregram **b**). Zone assignments: LE leading electrolyte, 1 tartaric acid, 2 formic acid, 3 citric acid, 4 malic acid, 5 lactic acid, 6 gluconic acid, 7 succinic acid, 8 acetic acid, TE terminating electrolyte

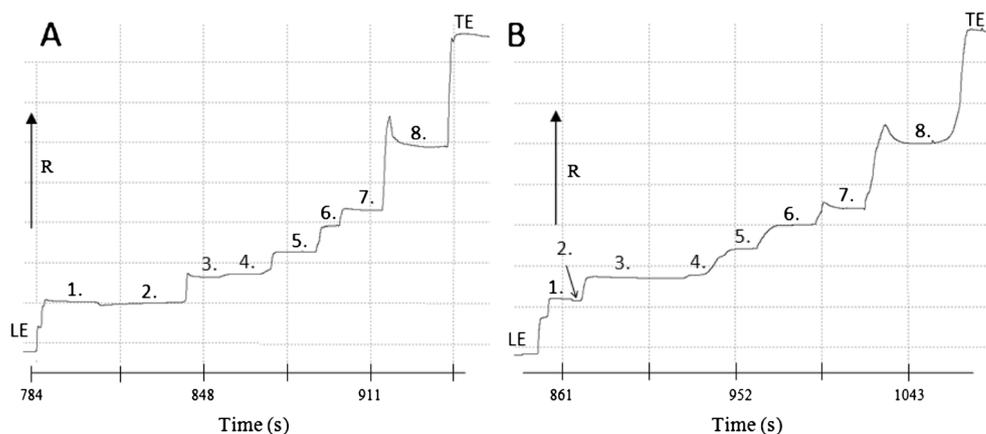


Table 1 Validation parameters for organic acids in mead samples obtained using isotachophoretic method

Acids	Concentration (mg L ⁻¹)		Method accuracy		Recovery studies (%)				Method precision	
					Mead worts				Mead worts	
					1:1		1:2		1:1	1:2
	Mead worts		LOD (mg L ⁻¹)	LOQ (mg L ⁻¹)	Added amount level 1 (50 %)	Added amount level 2 (25 %)	Added amount level 1 (50 %)	Added amount level 2 (25 %)	RSD (%)	
1:2 1:3										
Tartaric	273±2	<LOD	1.1	3.4	96±2	101±1	98±3	99±3	0.9	0.4
Formic	59±2	<LOD	2.6	7.8	99±3	100±1	98±3	97±1	2.0	1.3
Citric	1813±9	1265±2	3.8	11.4	98±3	99±1	102±1	101±1	0.9	0.2
Malic	271±5	348±3	0.7	2.2	100±1	99±1	100±1	97±1	2.5	1.0
Lactic	447±4	348±6	1.7	5.0	101±1	100±1	98±1	101±1	1.4	2.3
Gluconic	1058±6	549±3	4.7	14.2	99±1	100±2	96±0	99±0	1.2	1.1
Succinic	690±11	814±4	1.2	3.7	97±1	100±2	100±1	98±1	2.5	0.8
Acetic	611±13	473±4	4.7	14.2	97±1	97±3	99±1	101±1	1.7	0.7

submitted to measurement for five times. According to the validation protocol, the recovery values should fit the range 95–105 %. The limit of detection (LOD) and limit of quantification (LOQ) for each organic acid were calculated based on the residual standard deviation (SD) of the analytical signal and slope of the calibration curve(s), according to the formulas: LOD=3.3 SD/s, LOQ=10 SD/s, respectively.

Results and Discussion

The proposed isotachophoretic method allowed the isolation of eight acids in mead samples with satisfactory resolution. The isotachophoregrams of a standard mixture solution and the example mead sample are presented in Fig. 1. The analytical range was found to be linear from 3.125 to 100 mg L⁻¹ with high correlation coefficients of calibration curves $R > 0.998$. The calculated validation parameters were collected in Table 1. The recovery rates of all analytes were between 96 and 101 %. The obtained recovery range was comparable to this obtained by Dezmiorean et al. (2012), who determined organic acids in fermented products obtained from Romanian honey using HPLC method (95–105 %). The precision, which involved repeatability of analysis ($n=6$), was evaluated using a mead sample and spiked mead samples (as mentioned above). Relative standard deviation value of the zone length was equal or less than 2.5 %. The results were slightly higher than those reported by Dezmiorean et al. (01–09 %), but complied with validation criterion of precision. The isotachophoretic system showed also high sensibility due to low limits of detection and quantification. For all investigated organic acids, LOD ranged from 0.7 to 4.7 mg L⁻¹ and LOQ values ranged between 2.2 and 14.2 mg L⁻¹ whereas LOD and

LOQ determined by Dezmiorean et al. were in the range 0.50–8.32 and 1.62–18.25, respectively, but expressed in milligrammes per kilogramme.

The reliable and cheap isotachopheresis method with conductivity detector, described in this study, enabled to determine eight organic acids in a mead sample. All the validation parameters indicate high reliability of the method. Moreover, sample preparation is fast and simple, needing only dilution before injection. The proposed method makes possible to monitor changes in the composition profile and the concentration of organic acids during the mead wort fermentation, as well as it enables to control the maturity of the final product. Furthermore, it can be used to test commercial mead available in the market in order to detect the presence of organic acids from other sources than mead and fermentation process (e.g. from fruit juices or organic acid additives).

Compliance with Ethics Requirements

Conflict of Interest Justyna Dobrowolska-Iwanek declares that she has no conflict of interest. This article does not contain any studies with human or animal subjects.

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