Journal of Chromatography A, 1202 (2008) 19–33

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

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

ARTICLE INFO

Article history:
Received 16 February 2008
Received in revised form 29 May 2008
Accepted 12 June 2008
Available online 18 June 2008

Keywords:
Mead
Honeywine
Phenolic compound
Antioxidant
CoulArray detector
HPLC

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).

0021-9673/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2008.06.016

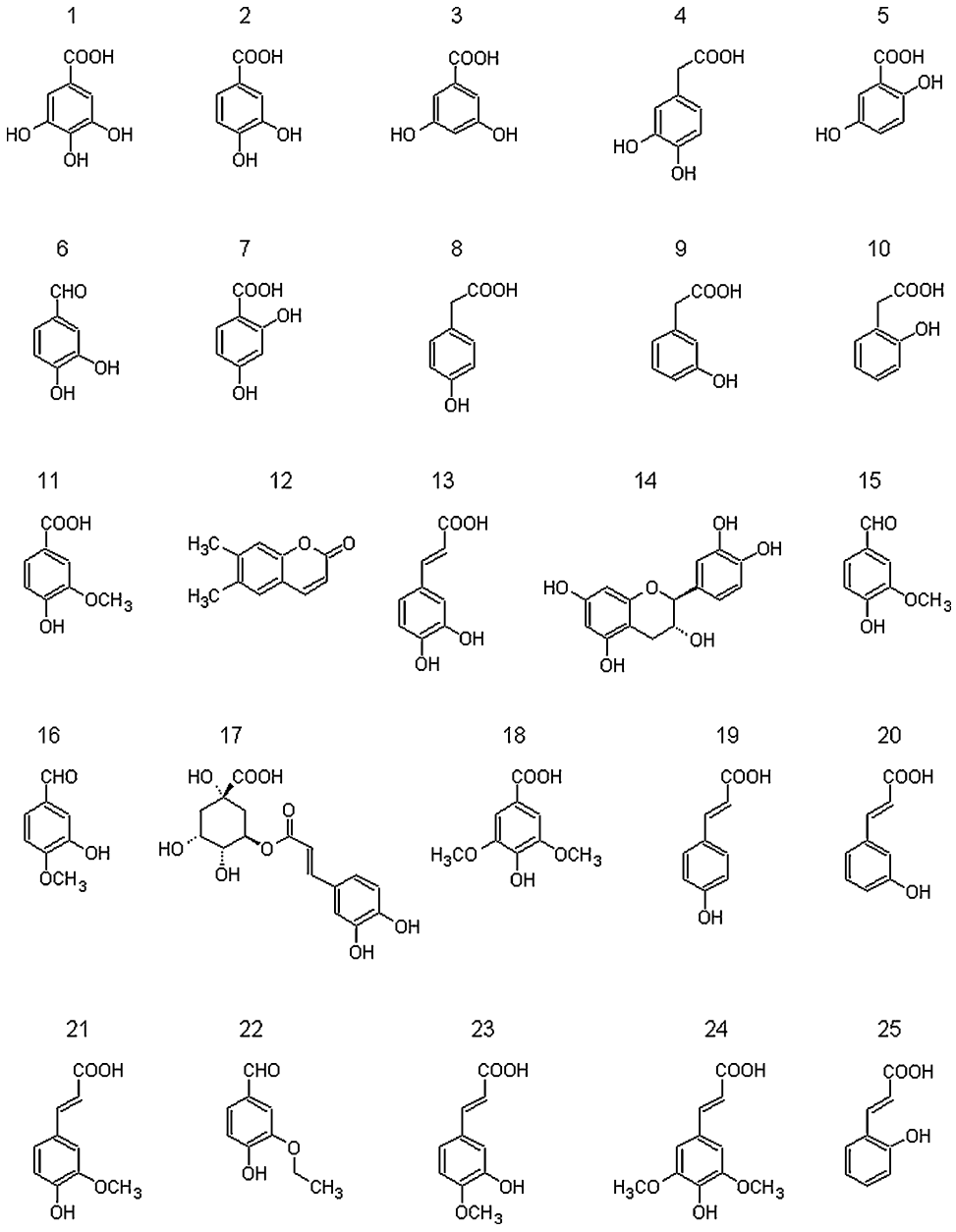

Fig. 1. Structures of the phenolic compounds.

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.

2. Experimental

2.1. Chemicals

Standard phenolic compounds (HPLC purity) were obtained from the following sources. 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) were purchased from Fluka (Buchs, Switzerland). 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) were purchased from Sigma–Aldrich (St. Louis, USA). Structural formulas of phenolic compounds are shown in Fig. 1. Standards of the phenolic compounds were dissolved in aqueous methanol (1:1, v/v) to obtain 100 mg/L stock solutions and filtered through the 0.2 μm PTFE filter. Stock solutions were stored in dark brown glass vials at 4 °C in darkness for 1 week at maximum. Calibration solutions ranging from 0.025 to 2.5 mg/L were prepared by dilution of stock solutions with the mobile phase A in the desired volume ratios and 10 μl was immediately injected to HPLC.

Hydroxymethylfurfural (HPLC purity) was obtained from Sigma–Aldrich. Standard of hydroxymethylfurfural was dissolved in aqueous methanol (9:1, v/v) to obtain 400 mg/L stock solution and filtered through the 0.2 μm PTFE filter. Stock solutions were stored in dark brown glass vials at 4 °C in darkness for 1 week at maximum. Calibration solutions ranging from 0.5 to 18 mg/L were prepared by dilution of stock solutions with the aqueous methanol (9:1, v/v) in the desired volume ratios and 10 μl was immediately injected to HPLC. Acetonitrile and methanol, LiChrosolv gradient grade, was obtained from Merck (Darmstadt, Germany). Formic acid (98–100%) was obtained from Sigma–Aldrich. Ammonium acetate (99.995%) was obtained from Fluka. Water was distilled in glass and purified using a Mili-Q water purification system (Milipore, Bedford, MA, USA).

2.2. Mead samples

Mead samples were diluted with the initial mobile phase at 1:1 to 1:50 (depending on the concentration), filtered through the 0.2 μm PTFE filter only and 10 μl was immediately injected to HPLC. No other sample pre-treatments were used. All the mead samples were obtained from the Czech trade network from the following manufacturers: 1, JANKAR PROFI, s.r.o.; 2, Česká včela, s.r.o.; 3, an unknown (distributor: Berentzen Distillers CR, spol s.r.o.); 4, Medoprodukt, s.r.o.; 5, Rajmund Krátký; 6, Ing. Marek Sznapka; 7, Včelnex, s.r.o.; 8, Dalibor Hromčík; 9, Hana Boháčová – APICOR; 10, Včela Předboj, a.s.; 11, Výzkumný ústav včelařský, s.r.o.; 12, Trikam, v.o.s.; 13, Lubomír Skřivánek; 14, Evžen Báchor.

2.3. Equipment

A HPLC system for phenolic compounds analysis was consisted of a vacuum degasser DG 3014 (Ecom, Prague, Czech Republic), two chromatographic pumps model 582 (ESA, Chelmsford, MA, USA) a CoulArray thermostatic organizer (ESA, Chelmsford, MA, USA) containing: a pulse damper, a gradient mixer, a manual injector with 10 μl sampling loop (Rheodyne, Cottati, CA, USA); an electrochemical 8-channel CoulArray 5600A detector (ESA Chelmsford, MA, USA) and a PC with a CoulArray software for data acquisition, processing and analysis (ESA Chelmsford, MA, USA). Chromatographic column Gemini C18 (150 mm × 3 mm I.D., 3 μm particle size) was obtained from Phenomenex (Torrence, CA, USA). Hold-up volume (0.59 mL) was determined using uracil as the non-retained marker solute.

Hydroxymethylfurfural analysis was carried out using HPLC system Hewlett-Packard model HP 1090M (Waldbronn, Germany) equipped with a diode array detector and an autosampler. Chromatographic column LiChrospher 60 RP-select B (250 mm × 4 mm I.D., 5 μm particle size) was obtain from Merck (Darmstadt, Germany). Hold-up volume (3.18 mL) was determined using uracil as the non-retained marker solute.

2.4. HPLC analysis of phenolic compounds

All chromatographic separations were carried out at 35 ± 0.1 °C using the gradient elution with mobile phases A and B. The mobile phase A was 5 mM ammonium acetate in water and pH value was adjusted to 3.0 by formic acid adding (1000 μl/L). The mobile phase B was a mixture of mobile phase A and acetonitril at 1:2 (v/v) and pH value was adjusted to 3.0 by formic acid addition (600 μl/150 mL). The mobile phases were filtered through the 0.45 μm filter and degassed (for 10 min) by ultrasonication before use. The applied gradient programme was: 0–27 min, 2% B isocratic; 27–72 min, linear gradient from 2 to 7% B; 72–108 min, linear gradient from 7 to 25% B; and finally, washing and reconditioning of the column was done. The flow rate was 0.45 mL/min and the injection volume was 10 μl. Electroactive compounds were monitored and quantified using eight electrochemical cells with applied potentials from 200 to 900 mV (100 mV increment).

2.5. HPLC analysis of hydroxymethylfurfural

All chromatographic separations of hydroxymethylfurfural were carried out at laboratory temperature using the isocratic elution with the mobile phase consisting of aqueous methanol (9:1). The mobile phase was filtered through the 0.45 μm filter and degassed (for 10 min) by ultrasonication before use. The flow rate was 1.0 mL/min and the injection volume was 10 μl. Hydroxymethylfurfural was determined using diode array UV detector at 285 nm.

3. Results and discussion

3.1. Optimization of separation conditions for the analysis of phenolic compounds

The chromatographic separation of phenolic compounds strongly depends on the pH of the mobile phase. Results of previous study aimed on the optimization of the HPLC analysis of the phenolic compounds have confirmed that the mobile phase acidity approx. 3.14–3.47 is the best for separation [31]. The optimization procedure was focused on the mobile phase acidity, the gradient steepness and the flow rate.

The first part was focused on the selection of the suitable mobile phase. Eight least-retained phenolic compounds (1–8) were used for the optimization due to many polar electroactive compounds present in mead samples. An unknown electroactive impurity from the mobile phase had to be added in optimization procedure, too. Five values of the mobile phase acidity were tested: 2.80, 2.90, 3.00, 3.15 and 3.35. Five isocratic separations with the different elution strength from 2 to 10% B (2% B increment) were carried out for each mobile phase. The flow rate used in optimization was 0.40 mL/min. Because of high compounds similarity and complex matrix, a minimum required resolution had to be chosen at $R_s \geq 1.0$ as criterion of sufficient separation. The most suitable mobile phase was pH 3.35. All compounds of interest were best resolved at 10 and 8% B. However, when applied the method to mead samples, protocatechuic acid and gallic acid coeluted with unknown electroactive compounds. Each mobile phase was then applied to mead sample. Using step-by-step procedure, the optimal mobile phase with pH 3.00 and 2% B was selected. This mobile phase was used as the initial composition in the gradient programme. The full results expressed as the influence of the mobile phase acidity and acetonitrile concentration on the resolution are summarised in Table 1.

On the basis of preliminary analysis using the linear gradient programme (0–100 min, 0–50% B) three analyte groups difficult to separate (2–6, 12–18 and 21–25) were found out. Hence, the proposed gradient programme consisted of three parts, the first part is isocratic, the second and third parts are gradient. The first isocratic part was 2% B for 30 min. The steepness of the second gradient part (30–80 min) was reduced from 0.3% (0.017% B/min decrease) and steepness of the third gradient part (80–120 min) was reduced from 0.75% B/min (0.125% B/min decrease) to obtain the required resolution. The best resolution was obtained under the following final conditions: 0–30 min, 2% B isocratic; 30–80 min, the linear gradient from 2% to 8% B; 80–120 min, the linear gradient from 8 to 25% B. The gradient programme was successfully applied to the analysis of mead samples and no coelutions were found.

The last optimization step was the finding of the maximal flow rate what the backpressure allows for the shortest analysis. The flow rate was increased from 0.40 mL/min with 0.05 mL/min increment. The highest acceptable flow rate for long-time trouble-free operation is 0.45 mL/min. The chromatogram of the separation of standard phenolic compounds measured at optimal separation conditions is shown in Fig. 3.

3.2. Qualitative analysis of phenolic compounds

The determination of phenolic compounds with available standards was carried out by comparing the chromatograms of standard mixtures, the sample and sample spiked with the standard mixture. The peak identity confirmation was done by the comparison of both retention times and the voltametric behaviour across the coulometric-array with those obtained by injecting standards measured at the same conditions. Each compound has a specific voltametric fingerprint. The peak identity was confirmed if following conditions were fulfilled: the number of electrochemical signals and their peak ratios of an unknown compound agreed with the data of appropriate standard phenolic compound. The ratios of the area of the dominant peak (the channel providing the highest response) to the area of so-called pre-dominant and the post-dominant peak (the channel just before or just the dominant peak channel, respectively) were used for the determination of appropriate compound. Electrochemical characteristics of the phenolic compounds measured at optimal separation conditions are shown in Table 2.

Table 1
Optimization of separation conditions—map of resolution

Mobile phase acidity	pH 2.80					pH 2.90					pH 3.00					pH 3.15					pH 3.35				
Mobile phase B (%)	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10
Critical pair	Resolution																								
Impurity 1	4.74	1.49	1.11	2.69	3.98	4.57	1.42	1.11	2.63	3.94	4.42	1.31	1.11	2.48	3.78	4.08	1.30	1.10	2.53	3.90	3.77	1.08	1.21	2.52	3.56
1–2	19.7	18.1	14.4	14.7	12.9	19.4	18.0	14.3	14.0	14.1	19.7	17.9	9.19	13.3	12.9	15.3	19.8	15.5	13.3	13.7	19.9	16.4	13.7	15.0	14.0
2–3	2.89	1.45	0.00	0.00	0.00	2.73	1.39	0.00	0.00	1.01	2.35	1.30	0.00	0.00	1.01	1.91	1.09	0.00	0.00	1.09	1.21	0.00	0.00	1.08	1.22
3–4	4.88	4.28	4.51	4.02	3.07	5.08	4.65	4.55	3.92	4.92	5.22	5.04	4.81	3.75	4.36	7.06	5.83	4.94	3.75	4.39	4.36	5.88	4.41	5.15	4.16
4–5	4.90	5.67	6.25	6.61	6.75	4.79	6.99	6.34	6.62	6.81	3.68	4.09	6.27	5.83	6.48	1.12	2.70	4.17	5.17	5.91	2.25	0.00	1.72	2.94	3.58
5–6	1.50	2.27	2.90	3.20	3.32	0.00	1.02	1.43	1.77	1.88	1.24	0.37	0.00	0.00	0.00	4.90	3.44	2.40	1.69	1.29	6.17	6.67	5.22	4.08	3.52
6–7	11.6	10.2	10.4	9.73	8.27	10.8	12.5	9.09	10.5	8.94	8.79	10.4	9.95	7.62	8.86	6.10	6.72	7.17	8.14	7.19	1.34	2.62	3.58	4.17	4.57
7–8	1.42	0.00	2.34	3.40	3.85	2.50	0.00	0.00	2.52	3.07	3.66	1.59	0.00	0.96	2.02	6.16	3.71	1.82	0.00	0.00	10.4	6.82	4.55	2.83	1.61

Table 2
Electrochemical characteristics of the phenolic compounds measured at optimal separation conditions

No.	Phenolic compound	Significant peaks		Peak area (%)								Identification based on peak area ratios			
		Dominant peak[a] (mV)	Measured peak[b] (mV)	200 mV	300 mV	400 mV	500 mV	600 mV	700 mV	800 mV	900 mV	Dominant peak location		Peak area size	
												Pre/Dom	Post/Dom	2nd/1st	3rd/1st
1	Gallic acid	400	400			25	22	14	18	16	5		0.87	0.87	0.71
2	Protocatechuic acid	900	500			1	24	11	11	16	37	0.42		0.65	0.42
3	α-Resorcylic acid	900	900								100				
4	Homoprotocatechuic acid	900	900			11	18	9	10	15	37	0.39		0.48	0.39
5	Gentisic acid	900	900		1	23	7	8	10	15	36	0.42		0.64	0.42
6	Protocatechuicaldehyde	500	500				29	14	12	17	28		0.49	0.95	0.57
7	β-Resorcylic acid	900	900								100				
8	4-Hydroxyphenylacetic acid	900	900							5	95	0.05		0.05	
9	3-Hydroxyphenylacetic acid	900	900								100				
10	2-Hydroxyphenylacetic acid	900	900							4	96	0.04		0.04	
11	Vanillic acid	900	800						2	47	51	0.94		0.94	0.04
12	Esculetin	900	900			1	30	8	10	14	38	0.36		0.81	0.36
13	Caffeic acid	900	900			24	12	8	10	14	32	0.43		0.74	0.43
14	(+)-Catechin	800	800			18	11	5	7	26	33	0.78		0.78	0.53
15	Vanillin	900	900							32	68	0.47		0.47	
16	Isovanillin	900	900							44	56	0.78		0.78	
17	Chlorogenic acid	900	900			20	14	6	8	12	40	0.29		0.51	0.34
18	Syringic acid	900	700					7	37	13	44	0.29		0.83	0.29
19	*p*-Coumaric acid	900	900							31	69	0.44		0.44	
20	*m*-Coumaric acid	900	900								100				
21	Ferulic acid	700	700					9	42	16	33	0.21	0.38	0.80	0.38
22	Ethylvanillin	900	800							47	53	0.87		0.87	
23	Isoferulic acid	900	900						34	23	43	0.54		0.78	0.54
24	Sinapic acid	600	600				10	59	19	11		0.17	0.33	0.33	0.19
25	*o*-Coumaric acid	900	900							15	85	0.17		0.17	

[a] Dominant peak, peak of a cell potential providing the highest response.

[b] Measured peak, peak of a cell potential used for quantitative analysis; Pre/Dom, ratio of the predominant peak (peak before dominant peak) to dominant peak; Post/Dom, ratio of the post-dominant peak (peak after dominant peak) to dominant peak; 2nd/1st, ratio of the second-largest peak to the largest peak (dominant peak); 3rd/1st, ratio of the third-largest peak to the largest peak (dominant peak).

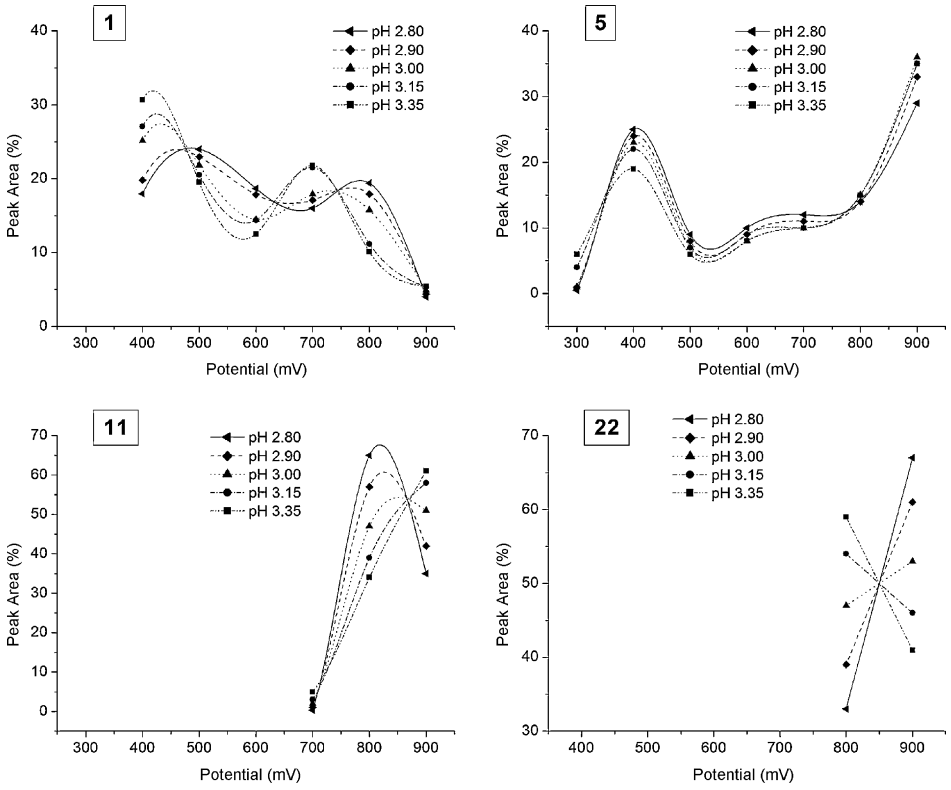

Fig. 2. Effect of the mobile phase acidity on the electrochemical detector response (peak areas) for: 1, gallic acid; 5, gentisic acid; 11, vanillic acid; 22, ethylvanillin.

3.3. *Detection conditions and quantitative analysis of phenolic compounds*

The potential selection significantly affects the selectivity, sensitivity and calibration parameters. Data from optimization of separation conditions confirm the influence of mobile phase pH on the voltametric behaviour across the coulometric-array. Measuring at the higher mobile phase pH provides higher sensitivity, lower noise interferences and increased response at lower potentials (Fig. 2). Higher mobile phase pH is suitable for the electrochemical detection, but the separation selectivity for phenolic compounds decreases. The potential selection for each phenolic compound has carried out according to the following conditions. The potential with the maximal response (peak area) was selected first. In case of partial coelution ($R_S < 1.0$), the following potential with the lower response but passing the separation condition ($R_S \geq 1.0$) was chosen. At different potentials, interfering compounds may provide significantly lower response or no response at all. In case of similar responses (at maximum 20% difference) at two potentials with the highest response, the potential with the lower voltage value was used. The drift to the lower voltage value increases the selectivity and decreases noise interferences as well. Due to this reason, the potential with the lower response were used for the quantification of vanillic acid and syringic acid (Table 2).

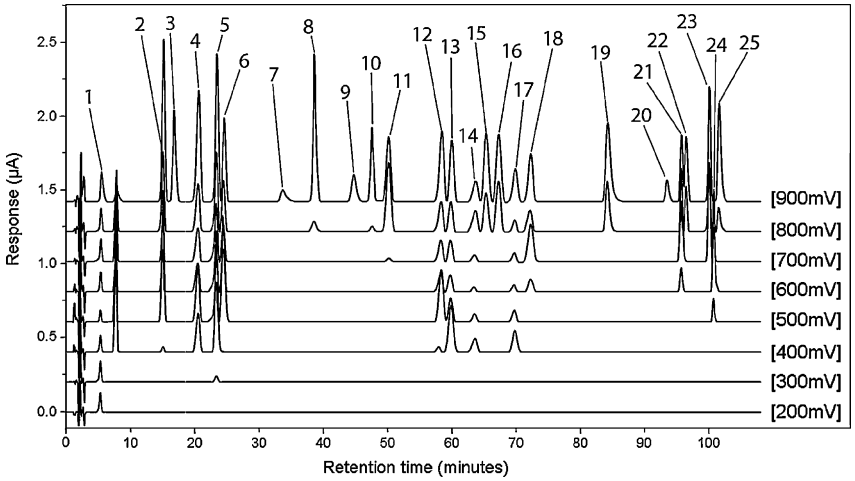

Fig. 3. Chromatographic separation of the phenolic compounds. Experimental conditions: Column Phenomenex Gemini C18 (150 mm × 3 mm I.D., 3 μm particle size), electrochemical detection 200–900 mV (with 100 mV increment), the flow rate 0.45 mL/min, the column temperature 35 °C, the injection volume 10 μl, the mobile phase gradient 0–27 min, 2% B isocratic; 27–72 min, linear gradient from 2 to 7% B; 72–108 min, linear gradient from 7 to 25% B.

Table 3
Parameters of calibration equations and validation characteristics of the phenolic compounds

No.	Phenolic compound	Retention time (min)	Concentration range (mg/L)	Slope (nC L/mg)	Intercept (nC)	Correlation coefficient (1)	LOD[a] (μg/L)	LOQ[b] (μg/L)	Precision						Accuracy		
									Intra-day			Inter-day					
									min[c] (%)	mid[d] (%)	max[e] (%)	min[c] (%)	mid[d] (%)	max[e] (%)	min[c] (%)	mid[d] (%)	max[e] (%)
1	Gallic acid	7.4	0.025–2.5	9 157 ± 30	21 ± 32	0.9999	4	12	3.35	1.17	1.38	4.85	1.84	1.81	97.4	100.8	98.4
2	Protocatechuic acid	15.2	0.025–2.5	10 677 ± 84	67 ± 88	0.9997	4	12	3.81	1.45	1.67	4.09	1.64	1.86	98.0	97.9	99.1
3	α-Resorcylic acid	16.9	0.025–1.0	21 037 ± 183	15 ± 86	0.9997	8	24	4.85	2.45	3.69	5.42	3.02	4.68	106.9	102.2	104.8
4	Homoprotocatechuic acid	20.2	0.025–1.0	14 174 ± 109	101 ± 51	0.9998	7	23	4.44	1.48	1.77	5.18	1.91	1.84	102.0	101.9	102.2
5	Gentisic acid	22.3	0.025–1.0	15 765 ± 138	75 ± 65	0.9997	6	19	4.61	0.92	1.61	4.90	1.50	2.16	103.0	99.3	100.2
6	Protocatechuicaldehyde	24.0	0.025–2.5	13 647 ± 69	−127 ± 72	0.9999	5	14	3.53	1.05	2.18	4.28	1.73	2.32	97.7	100.1	98.2
7	β-Resorcylic acid	33.2	0.1–2.5	7 841 ± 108	81 ± 133	0.9994	23	75	4.34	2.83	3.24	5.28	3.15	4.60	103.4	101.2	103.7
8	4-Hydroxyphenylacetic acid	37.9	0.05–2.5	25 530 ± 130	−7 ± 147	0.9999	9	27	1.94	0.91	1.84	3.14	1.36	2.48	99.7	101.3	99.6
9	3-Hydroxyphenylacetic acid	44.4	0.05–2.5	12 980 ± 119	−93 ± 133	0.9997	14	46	3.74	1.18	1.90	4.98	1.62	2.94	105.0	102.1	100.6
10	2-Hydroxyphenylacetic acid	47.0	0.05–2.5	14 379 ± 215	85 ± 241	0.9991	10	31	4.30	1.92	1.44	5.14	2.05	2.38	104.1	97.0	100.3
11	Vanillic acid	49.7	0.05–2.5	9 468 ± 82	71 ± 92	0.9997	12	39	2.46	1.58	1.02	3.94	1.94	1.41	98.9	98.3	99.1
12	Esculetin	58.0	0.05–2.5	10 891 ± 141	−39 ± 158	0.9993	14	45	3.82	1.92	2.28	5.17	2.40	3.24	98.3	102.3	99.3
13	Caffeic acid	59.4	0.05–2.5	8 696 ± 68	43 ± 76	0.9998	14	46	3.58	1.99	1.90	3.91	3.31	2.29	99.4	98.4	96.0
14	(+)-Catechin	63.1	0.1–2.5	4 170 ± 66	93 ± 81	0.9993	29	94	3.58	1.60	2.76	5.03	2.57	3.83	103.9	98.8	96.5
15	Vanillin	64.9	0.05–2.5	10 920 ± 46	58 ± 52	0.9999	11	36	2.95	0.93	1.77	3.71	1.18	2.43	98.2	100.6	101.9
16	Isovanillin	66.8	0.05–2.5	13 032 ± 72	52 ± 80	0.9999	12	37	2.85	1.29	1.71	3.61	1.62	2.67	97.7	101.7	100.3
17	Chlorogenic acid	69.1	0.1–2.5	5 592 ± 50	37 ± 61	0.9998	24	77	3.20	1.80	2.43	4.15	3.69	3.59	105.2	98.6	102.8
18	Syringic acid	71.5	0.05–2.5	6 960 ± 76	−62 ± 85	0.9995	14	45	3.17	1.56	1.74	5.04	2.18	2.41	101.8	97.4	99.0
19	*p*-Coumaric acid	83.3	0.05–2.5	11 467 ± 136	153 ± 152	0.9994	11	35	4.08	1.71	2.37	5.47	2.20	3.77	101.9	102.6	103.6
20	*m*-Coumaric acid	93.2	0.05–2.5	10 091 ± 152	−277 ± 170	0.9991	15	49	5.10	2.13	3.90	6.83	3.46	5.23	104.1	103.8	103.1
21	Ferulic acid	95.5	0.025–2.5	7 708 ± 63	−74 ± 66	0.9997	6	17	4.38	1.48	1.90	4.64	1.94	2.91	98.4	98.9	100.6
22	Ethylvanillin	96.3	0.025–2.5	7 087 ± 70	28 ± 73	0.9995	6	19	3.82	2.82	2.85	4.15	4.46	3.32	104.3	103.6	102.7
23	Isoferulic acid	100.0	0.025–2.5	10 197 ± 56	99 ± 58	0.9999	7	22	3.38	2.40	1.78	5.04	3.66	2.30	105.7	98.2	99.1
24	Sinapic acid	100.7	0.025–2.5	7 176 ± 38	12 ± 39	0.9999	6	17	3.63	1.35	1.82	4.85	2.14	1.94	96.8	99.2	97.8
25	*o*-Coumaric acid	101.3	0.025–2.5	12 147 ± 166	−20 ± 173	0.9991	8	24	4.84	2.02	1.75	6.55	4.71	2.56	106.2	101.9	102.9

[a] LOD, limit of detection at the signal three times at the baseline noise.
[b] LOQ, limit of quantification at the signal ten times at the baseline noise.
[c] at the lowest calibration point.
[d] in the middle of calibration.
[e] at the highest calibration point.

Table 4
Phenolic compounds and hydroxymethylfurfural content in mead samples

	Manufacturer 1							Manufacturer 2		
	Mead sample no. 1 (type of mead: traditional; vintage: 2006; Other ingrediences: propolis (trace)[a])	Mead sample no. 2 (type of mead: traditional; vintage: 2007; Other ingredi-ences:propolis (trace)[a])	Mead sample no. 3 (type of mead: traditional; vintage: 2003 Other ingredients: (archival); propolis (trace)[a])	Mead sample no. 4 (type of mead: sour cherry; vintage: 2006; Other ingredients: sour cherry juice, propolis (trace)[a])	Mead sample no. 5 (type of mead: sour cherry; vintage: 2007; Other ingredients: sour cherry juice, propolis (trace)[a])	Mead sample no. 6 (type of mead: blackcurrant; vintage: 2006; Other ingredients: blackcurrant juice, propolis (trace)[a])	Mead sample no. 7 (type of mead: Ginseng; vintage: 2006; Other ingredients: Ginseng extract, propolis (trace)[a])	Mead sample no. 8 (type of mead: classical; vintage: 2003; Other ingredients: sugar, aroma, hop extract, citric acid, caramel[a])	Mead sample no. 9 (type of mead: herbal; vintage: uninitiated; sugar, aroma, hop extract, citric acid, caramel[a])	Mead sample no. 10 (type of mead: walnut; vintage: 2003; Other ingredients: sugar, aroma, hop extract, citric acid, caramel[a])
Alcohol (%)	15	15	15	15	15	15	15	18	12.5	18
Hydroxymethylfurfural (mg/L)	7.49	6.66	25.9	5.39	3.30	4.51	6.53	18.9	27.8	42.2
Phenolic compound (*c* (mg/L))										
1. Gallic acid	0.04	0.52	0.33	0.20	2.45	1.36	0.71	ND	ND	ND
2. Protocatechuic acid	0.19	0.26	0.69	2.11	1.02	1.37	0.24	0.07	0.15	0.06
3. α-Resorcylic acid										
4. Homoprotocatechuic acid						0.11				
5. Gentisic acid			0.15	0.23	0.62	0.77				
6. Protocatechuicaldehyde	<LOQ	<LOQ	ND	0.10	ND	0.05	<LOQ	<LOQ	<LOQ	<LOQ
7. β-Resorcylic acid										
8. 4-Hydroxyphenylacetic acid	0.19	0.16	ND	ND	0.16	ND	<LOQ	0.11	<LOQ	<LOQ
9. 3-Hydroxyphenylacetic acid										
10. 2-Hydroxyphenylacetic acid										
11. Vanillic acid	0.25	0.32	0.41	2.61	0.71	0.55	0.15	0.11	0.10	0.09
12. Esculetin										
13. Caffeic acid	0.69	0.43	0.45	2.61	6.38	5.05	0.15			<LOQ
14. (+)-Catechin										
15. Vanillin								1.75	2.67	2.21
16. Isovanillin										
17. Chlorogenic acid				8.87	0.45	0.59				
18. Syringic acid	0.19	0.17	0.10	0.19	0.23	0.20	<LOQ	<LOQ	0.12	<LOQ
19. *p*-Coumaric acid	0.67	<LOQ	0.16	0.75	1.61	0.34	ND	0.10		
20. *m*-Coumaric acid										
21. Ferulic acid	0.38	ND	ND	0.23	ND	0.56	ND	0.25	0.10	0.23
22. Ethylvanillin										
23. Isoferulic acid	<LOQ	0.10	ND	ND		<LOQ	ND	<LOQ	0.07	<LOQ
24. Sinapic acid				ND	0.47	0.51				
25. *o*-Coumaric acid										

Table 4 (*Continued*)

	Manufacturer 3								Manufacturer 4			
	Mead sample no. 11 (type of mead: monastical; vintage: 2005; propolis, tartaric acid, aromatic herbs[a])	Mead sample no. 12 (type of mead: monastical; vintage: 2006; Other ingredi-ences:propolis, tartaric acid, aromatic herbs[a])	Mead sample no. 13 (type of mead: mountain; vintage: 2005; Other ingredi-ences:propolis, tartaric acid, mountain herbs[a])	Mead sample no. 14 (type of mead: mountain; vintage: 2006; Other ingredi-ences:propolis, tartaric acid, mountain herbs[a])	Mead sample no. 15 (type of mead: almond; vintage: 2005; Other ingredi-ences:propolis, tartaric acid, almond extract[a])	Mead sample no. 16 (type of mead: almond; vintage: 2006; Other ingredi-ences:propolis, tartaric acid, almond extract[a])	Mead sample no. 17 (type of mead: herbal; vintage: 2005; Other ingredi-ences:propolis, tartaric acid, drug plants[a])	Mead sample no. 18 (type of mead: herbal; vintage: 2006; Other ingredi-ences:propolis, tartaric acid, drug plants[a])	Mead sample no. 19 (type of mead: old Slovak; vintage: 2005; Other ingredi-ences:aroma, tartaric acid[a])	Mead sample no. 20 (type of mead: herbal; vintage: 2005; Other ingredi-ences:aroma, tartaric acid[a])	Mead sample no. 21 (type of mead: almond; vintage: 2005; Other ingredi-ences:aroma, tartaric acid[a])	Mead sample no. 22 (type of mead: walnut; vintage: 2006; Other ingredi-ences:aroma, tartaric acid[a])
Alcohol (%)	11.5	11.5	11.5	11.5	11.5	11.5	13.5	13.5	13	13	13	13
Hydroxymethylfurfural (mg/L)	10.1	6.85	10.6	7.65	13.4	5.92	15.8	10.8	25.8	29.9	26.7	62.2
Phenolic compound (*c* (mg/L))												
1. Gallic acid	ND	5.51	3.23	6.20	1.95	6.63	2.86	6.28	ND	0.19	ND	ND
2. Protocatechuic acid	3.08	1.78	2.95	2.04	2.09	2.06	2.99	2.36	0.30	0.47	0.32	0.71
3. α-Resorcylic acid												
4. Homoprotocatechuic acid	0.21		0.19		0.08		0.32					
5. Gentisic acid												
6. Protocatechuicaldehyde	ND	ND	ND	ND	ND	ND	ND	ND				
7. β-Resorcylic acid												
8. 4-Hydroxyphenylacetic acid	1.26	1.03	1.00	1.18	1.09	1.06	1.53	1.19	0.07	0.14	0.13	0.08
9. 3-Hydroxyphenylacetic acid	ND		ND		ND		ND					
10. 2-Hydroxyphenylacetic acid												
11. Vanillic acid	2.12	1.49	2.15	1.84	1.43	1.33	2.31	1.91	0.15	0.16	0.21	0.16
12. Esculetin												
13. Caffeic acid	4.68	2.46	5.02	3.10	3.44	2.70	4.91	3.53	0.37	0.42	0.29	0.28
14. (+)-Catechin		0.58		0.44		0.45		0.59				
15. Vanillin	12.7	21.0	12.2	19.7	12.9	26.8	13.4	34.2				0.47
16. Isovanillin												
17. Chlorogenic acid	3.71	0.73	5.02	1.36	14.1	0.49	4.42	1.18	<LOQ	<LOQ	<LOQ	<LOQ
18. Syringic acid	0.46	0.23	0.43	0.40	0.44	0.41	0.62	0.42	<LOQ	<LOQ	<LOQ	<LOQ
19. *p*-Coumaric acid	2.60	1.53	2.42	1.96	2.09	1.75	4.43	2.03	0.13	0.23	0.21	<LOQ
20. *m*-Coumaric acid												
21. Ferulic acid	1.37	1.11	1.23	1.17	0.68	1.16	1.22	1.45	ND	ND	0.32	ND
22. Ethylvanillin					0.09				ND	ND	4.20	
23. Isoferulic acid	1.41	0.61	1.36	0.76	0.75	0.60	1.33	0.74	0.06	0.11	<LOQ	<LOQ
24. Sinapic acid												
25. *o*-Coumaric acid												

Table 4 (*Continued*)

	Manufacturer 5								Manufacturer 6			
	Mead sample no. 23 (type of mead: natural; vintage: 2005; Other ingrediences: herbs[a])	Mead sample no. 24 (type of mead: gold; vintage: 2005; Other ingrediences: Carpathian herbs[a])	Mead sample no. 25 (type of mead: classical; vintage: 2005; Other ingrediences: herbs[a])	Mead sample no. 26 (type of mead: coconut; vintage: 2005; Other ingrediences: herbs, coconut[a])	Mead sample no. 27 (type of mead: plum; vintage: 2005; Other ingrediences: herbs, plum[a])	Mead sample no. 28 (type of mead: blueberry; vintage: 2005; Other ingrediences: herbs, blueberry[a])	Mead sample no. 29 (type of mead: sour cherry; vintage: 2005; Other ingredi-ences:herbs, sour cherry[a])	Mead sample no. 30 (type of mead: apple; vintage: 2005; Other ingrediences: herbs, apple[a])	Mead sample no. 31 (type of mead: Celtic; vintage: 2005; Other ingrediences: spices, herbs[a])	Mead sample no. 32 (type of mead: raspberry; vintage: 2005; Other ingrediences: raspberry juice[a])	Mead sample no. 33 (type of mead: sour cherry; vintage: 2005; Other ingredi-ences:sour cherry juice[a])	Mead sample no. 34 (type of mead: bitter & almond; vintage: 2005; Other ingrediences: sugar, spices, caramel, aroma[a])
Alcohol (%)	11	11	11	11	11	11	11	11	11.6	14	14	14
Hydroxymethylfurfural (mg/L)	26.2	15.5	18.7	28.3	29.4	31.5	32.7	31.4	2.74	3.94	9.09	76.4
Phenolic compound (*c* (mg/L))												
1. Gallic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.05	ND	0.25
2. Protocatechuic acid	0.10	0.20	0.25	0.21	0.34	1.45	0.23	0.16	0.29	2.13	2.13	0.71
3. α-Resorcylic acid												
4. Homoprotocatechuic acid						<LOQ						0.09
5. Gentisic acid						0.06				0.09	ND	0.15
6. Protocatechuicaldehyde	ND	0.04	<LOQ	<LOQ	<LOQ	0.03	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.12
7. β-Resorcylic acid												
8. 4-Hydroxyphenylacetic acid	0.12	0.12	0.14	0.11	0.11	0.14	0.14	0.09	0.25	0.82	ND	0.32
9. 3-Hydroxyphenylacetic acid	<LOQ	0.11	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	ND	0.12
10. 2-Hydroxyphenylacetic acid												
11. Vanillic acid	0.25	0.22	0.26	0.39	0.25	0.42	0.24	0.20	0.23	0.87	2.34	0.35
12. Esculetin		<LOQ	<LOQ	<LOQ	<LOQ	0.12	<LOQ					<LOQ
13. Caffeic acid		<LOQ	0.20	0.32	0.27	0.45	0.22	0.43	1.40	1.84	5.34	1.17
14. (+)-Catechin											4.04	
15. Vanillin	<LOQ	0.13	0.23	8.53	<LOQ	<LOQ	0.58					0.12
16. Isovanillin												
17. Chlorogenic acid											8.27	0.23
18. Syringic acid	<LOQ	<LOQ	0.12	0.11	0.14	0.13	0.14	0.11	0.16	0.25	1.80	0.25
19. *p*-Coumaric acid	0.37	0.24	0.39	0.31	0.28	0.87	0.44	0.54	1.02	0.38	0.66	0.31
20. *m*-Coumaric acid												
21. Ferulic acid	0.18	0.10	0.29	0.18	0.29	0.92	0.27	0.25	0.57	0.44	ND	0.30
22. Ethylvanillin		0.71	0.07	1.23	3.15	0.07	0.21	0.05			ND	0.58
23. Isoferulic acid	0.25	0.15	0.36	0.40	0.21	0.13	0.41	0.26				0.22
24. Sinapic acid									0.08	0.26	0.45	0.10
25. *o*-Coumaric acid												

Table 4 (*Continued*)

	Manufacturer 7						Manufacturer 8			Manufacturer 9		Manufacturer 10
	Mead sample no. 35 (type of mead: Carpathian; vintage: 2004; Other ingredi-ences:herbs, spices[a])	Mead sample no. 36 (type of mead: natural; vintage: 2005; Other ingredi-ences:herbs (15 types), spices[a])	Mead sample no. 37 (type of mead: herbal; vintage: 2005; Other ingredi-ences:herbs (22 types), spices (2 types)[a])	Mead sample no. 38 (type of mead: walnut; vintage: 2005; Other ingredi-ences:herbs (17 types), spices, walnut extract[a])	Mead sample no. 39 (type of mead: Cinnamonic; vintage: 2003; Other ingredi-ences:herbs (18 types), spices, cinna-monic extract[a])	Mead sample no. 40 (type of mead: almond; vintage: 2005; Other ingredi-ences:herbs (18 types), spices, almond extract[a])	Mead sample no. 41 (type of mead: bitter; vintage: 2005; Other ingredi-ences:tartaric acid, herbs extract, caramel[a])	Mead sample no. 42 (type of mead: almond; vintage: 2005; Other ingredi-ences:tartaric acid, caramel, herbs extract, almond extract[a])	Mead sample no. 43; (type of mead: sour cherry; vintage: 2005; Other ingredi-ences:tartaric acid, caramel, herbs extract, sour cherry juice[a])	Mead sample no. 44 (type of mead: classical; vintage: 2005; Other ingredi-ences:sugar, herbs extracts, citric acid, caramel[a])	Mead sample no. 45 (type of mead: sour cherry; vintage: 2005; Other ingredi-ences:sugar, herbs extracts, citric acid, caramel[a])	Mead sample no. 46 (type of mead: old Czech balm; vintage: 2005; Other ingredi-ences:caramel, citric acid, herbs extract, spices extract[a])
Alcohol (%)	18	18	20	18	14	14	12	12	12	18	18	14.5
Hydroxymethylfurfural (mg/L)	15.5	122	113	150	158	99.0	44.4	45.1	37.6	47.6	35.0	60.6
Phenolic compound (*c* (mg/L))												
1. Gallic acid	ND	ND	ND	0.12	ND	ND	<LOQ	0.05	0.10	ND	ND	0.20
2. Protocatechuic acid	0.1	0.25	0.18	0.56		0.34	0.12	0.15	0.98	0.35	0.25	0.58
3. α-Resorcylic acid												
4. Homoprotocatechuic acid				0.06		<LOQ				<LOQ	<LOQ	
5. Gentisic acid				0.08		0.06			0.61		<LOQ	
6. Protocatechuicaldehyde	<LOQ	0.05	<LOQ	0.09	<LOQ	0.06	<LOQ	<LOQ	ND	<LOQ	<LOQ	<LOQ
7. β-Resorcylic acid												
8. 4-Hydroxyphenylacetic acid	0.10	0.11	0.10	0.14	<LOQ	0.08	ND	<LOQ	ND	0.21	ND	0.35
9. 3-Hydroxyphenylacetic acid												
10. 2-Hydroxyphenylacetic acid												
11. Vanillic acid	<LOQ	<LOQ	<LOQ	0.11		<LOQ	0.08	0.14	0.62	0.39	0.27	0.22
12. Esculetin			<LOQ	<LOQ								
13. Caffeic acid	<LOQ	<LOQ		<LOQ		<LOQ	<LOQ	<LOQ	0.31	0.31	0.42	0.30
14. (+)-Catechin												
15. Vanillin	<LOQ		3.10	15.2	0.13	0.15	4.64	54.8	3.23	2.89	2.43	
16. Isovanillin												
17. Chlorogenic acid							<LOQ		1.87			0.30
18. Syringic acid	<LOQ			<LOQ		<LOQ	0.13	0.15	<LOQ	<LOQ	<LOQ	0.12
19. *p*-Coumaric acid	<LOQ	0.11	0.14	0.11	0.08	0.10				1.31	0.94	0.46
20. *m*-Coumaric acid												
21. Ferulic acid	0.09	0.14	0.13	0.22	0.06	0.15	0.04	0.05	0.04	0.51	0.47	ND
22. Ethylvanillin	0.39	0.29	0.11	0.47	<LOQ	31.0	0.16	0.92	2.30		0.88	ND
23. Isoferulic acid	0.05	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ				0.15	0.10	<LOQ
24. Sinapic acid												
25. *o*-Coumaric acid												

Table 4 (*Continued*)

	Manufacturer 11	Manufacturer 12	Manufacturer 13	Manufacturer 14
	Mead sample no. 47 (type of mead: bitter; vintage: 2005; Other ingrediences:wine, sugar, herbs, aroma[a])	Mead sample no. 48 (type of mead: bitter; vintage: 2004; Other ingrediences:wine, sugar, caramel, aroma[a])	Mead sample no. 49 (type of mead: archival (3 yrs); vintage: uninitiated; caramel, herbs extract[a])	Mead sample no. 50 (type of mead: classical; vintage: 2006; Other ingrediences:herbs, spices[a])
Alcohol (%)	18	18	11	20
Hydroxymethylfurfural (mg/L)	49.1	62.0	139	4.82
Phenolic compound (*c* (mg/L))				
1. Gallic acid	ND	1.18	0.09	0.08
2. Protocatechuic acid	0.41	1.13	0.41	1.14
3. α-Resorcylic acid				
4. Homoprotocatechuic acid				
5. Gentisic acid				0.10
6. Protocatechuicaldehyde	<LOQ	ND	0.04	ND
7. β-Resorcylic acid				
8. 4-Hydroxyphenylacetic acid	0.23	0.22	ND	0.40
9. 3-Hydroxyphenylacetic acid				
10. 2-Hydroxyphenylacetic acid				
11. Vanillic acid	0.24	0.34	0.27	0.59
12. Esculetin			<LOQ	
13. Caffeic acid	1.55	1.26	0.91	3.38
14. (+)-Catechin				
15. Vanillin				
16. Isovanillin				
17. Chlorogenic acid	0.25	0.17	1.11	
18. Syringic acid	0.17	0.20	<LOQ	
19. *p*-Coumaric acid	0.74	0.40	0.33	10.6
20. *m*-Coumaric acid				
21. Ferulic acid	0.67	0.22	0.48	3.74
22. Ethylvanillin	ND			
23. Isoferulic acid	0.22	<LOQ	<LOQ	0.58
24. Sinapic acid				
25. *o*-Coumaric acid				

[a] Other declared ingredients (except for honey and water; *c*, concentration; <LOQ, detected but not determined; ND, not determined because of coelution with an unknown electroactive compound).

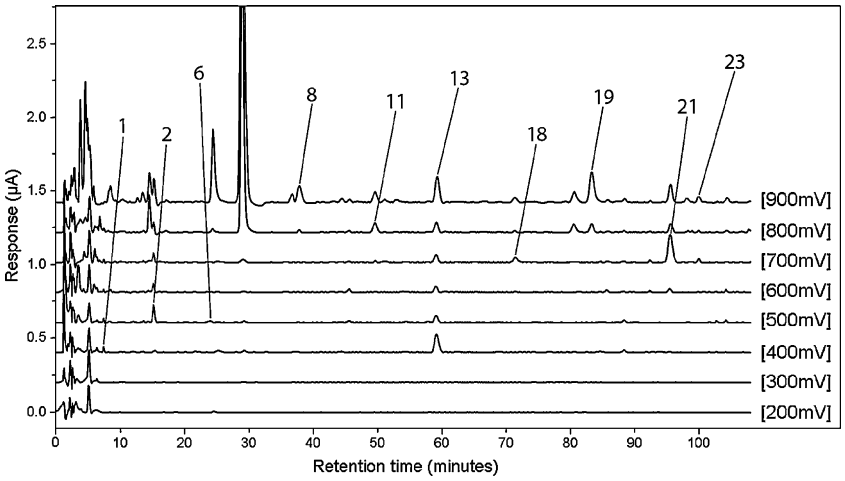

Fig. 4. Chromatographic separation of traditional mead (sample no. 1). All conditions are identical as for Fig. 3.

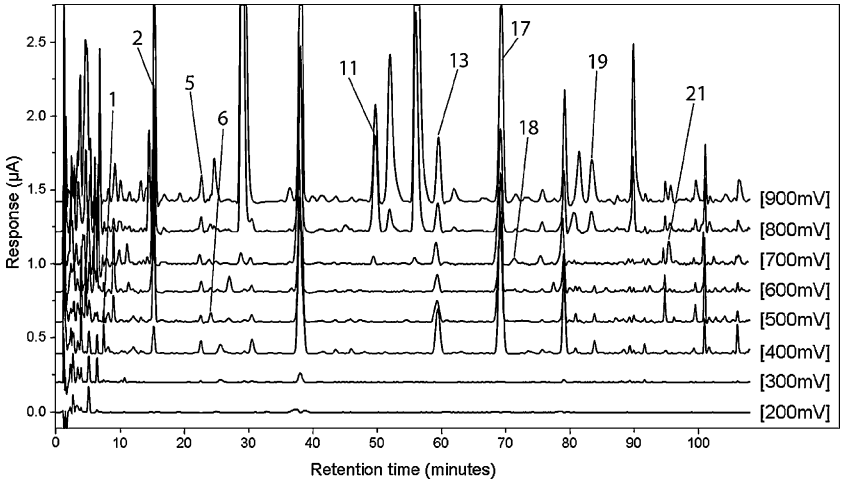

Fig. 5. Chromatographic separation of cherry sour mead (sample no. 4). All conditions are identical as for Fig. 3.

The quantitative analysis of the phenolic compounds was performed by the linear calibration curves including 5–7 calibration points. Peak areas were plotted versus theoretical concentrations and calibration curves were obtained from least-squares regression analysis. Limits of detection (LOD) were defined as 3 times signal-to-noise ratio (S/N) and limits of quantification (LOQ) were defined as 10 times signal-to-noise ratio (S/N). LODs were ranging from 4 to 29 μg/L and LOQs from 12 to 94 μg/L. Accuracy and precision of phenolic compounds were evaluated with sample no. 1 spiked with standards. Final values of concentrations of phenolic

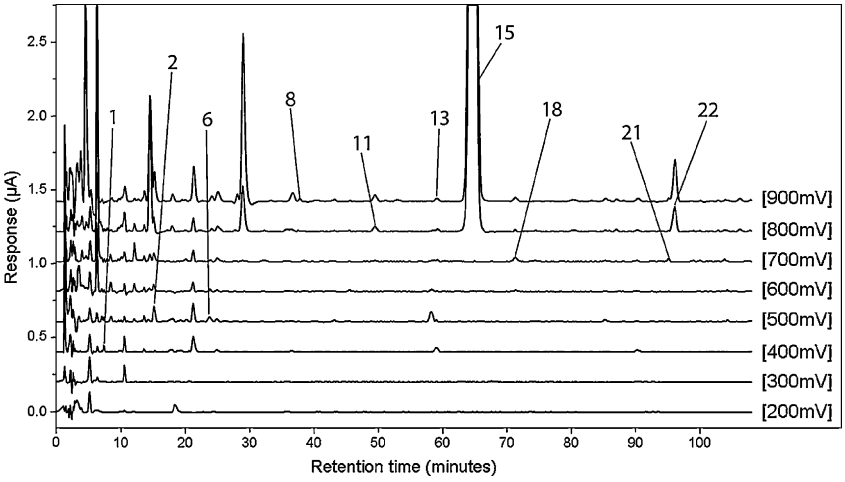

Fig. 6. Chromatographic separation of almond mead (sample no. 42). All conditions are identical as for Fig. 3.

standards present in spiked sample no. 1 were at the lowest calibration points (close to the LODs), in the middle of calibration and at the highest calibration points. The precision was expressed as the intra-day and inter-day relative standard deviation (RSD, %). Inter-day precision was performed in five replicate injections on the same day. Intra-day precision was performed in five replicate injections over 5-day period. The accuracy was expressed as the mean of measured value to the theoretical value (%). Parameters of calibration equations and validation characteristics of phenolic compounds are listed in Table 3.

3.4. Phenolic compounds in mead samples

Twenty-five phenolic compounds were analysed in 50 mead samples obtained from the Czech trade network. Phenolic compounds were divided into five groups: benzoic acid hydroxyderivatives, cinnamic acid hydroxyderivatives, phenylacetic acid hydroxyderivatives, sensorically significant compounds and other phenolic compounds. The qualitative and quantitative concentration of each determined phenolic compound was collated with meads composition and hydroxymethylfurfural concentration. Characteristic markers of added ingredients were searched. Clear evidence of a significant influence of a fruit juice addition on electroactive compounds content is evident from Figs. 4 and 5. Table 4 (parts 1–5) shows detailed results of the concentrations of all phenolic compounds and hydroxymethylfurfural in all studied samples together with the information of added ingredients provided by manufacturers. Table 5 summarised the typical of concentration ranges of studied compounds in 50 studied mead samples. This table is the most important output of this work.

Benzoic acid hydroxyderivatives were the most common phenolic compounds. The most frequently occurring phenolic acids were protocatechuic acid and vanillic acid (both of them were presented in 98% samples). These acids were not detected in the sample containing the highest amount of hydroxymethylfurfural (sample no. 39). High concentration of hydroxymethylfurfural and the absence of the most common phenolic compounds can be considered as an indicator of excessive heating during the mead production. The effect of heat treatment on phenolic compounds and hydroxymethylfurfural concentration in meads is being further studied in our laboratory. Other determined compounds were syringic acid (92%), gallic acid (50%) and gentisic acid (24%). α- and β-resorcylic acids were not detected in any sample.

Cinnamic acid hydroxyderivatives is a group of phenolic compounds occurring in the highest concentrations—chlorogenic acid up to 14.1 mg/L (sample no. 15) and *p*-coumaric acid up to 10.6 mg/L (sample no. 50). The most common representative of this group was caffeic acid (90%), *p*-coumaric acid (88%), ferulic acid (82%) and isoferulic acid (80%). Sinapic acid was detected in 12% samples, mainly in meads with added fruit juices (samples no. 5, 6, 32, 33). *m*- and *o*-coumaric acids were not detected at all.

Phenolic acid hydroxyderivatives were not observed so often as previous two groups. 4-Hydroxyphenylacetic acid was the most typical (84%) and detected in the highest concentration levels (1.53 mg/L, sample no. 17). 3-Hydroxyphenylacetic acid and homoprotocatechuic acid were detected in 22% samples only; 2-hydroxyphenylacetic acid was not detected.

Very important group is formed by sensorically significant compounds—vanillin and ethylvanillin. These compounds significantly influence smell and they are often added by manufacturers due to the adjusting of an undesirable meads bouquet. Vanillin (detected in 60% samples) originates from honey or propolis, but natural concentrations in meads should be in a trace level only, but higher concentration shows at a deliberate adding. Ethylvanillin (detected in 42% samples) is not of natural origin and each detection proofs the addition of this synthetic compound. The high amount of vanillin (54.8 mg/L) and ethylvanillin (31.0 mg/L) was detected in

Table 5
Summary of phenolic compounds and hydroxymethylfurfural concentration in mead samples

Type of compound	No.	Compound	Occurrence[a] (%)	Compound concentration (mg/L)			RSD range (%)
				Range (mg/L)	Average (mg/L)	Median (mg/L)	
Toxic		Hydroxymethylfurfural	100	2.74–157	37.4	26.5	0.07–2.84
Benzoic acid hydroxyderivatives	1	Gallic acid	50	0.04–6.63	1.73	0.61	0.19–4.21
	2	Protocatechuic acid	98	0.06–3.08	0.87	0.41	0.21–3.04
	5	Gentisic acid	24	0.06–0.77	0.27	0.15	0.56–3.98
	11	Vanillic acid	98	0.08–2.61	0.66	0.27	0.18–4.54
	18	Syringic acid	92	0.10–1.80	0.29	0.19	0.86–4.40
	3	α-Resorcylic acid	0				
	7	β-Resorcylic acid	0				
Cinnamic acid hydroxyderivatives	13	Caffeic acid	90	0.15–6.38	1.81	0.91	0.30–4.50
	17	Chlorogenic acid	46	0.16–14.1	2.95	1.15	0.47–3.40
	19	*p*-Coumaric acid	88	0.08–10.6	1.08	0.44	0.44–4.92
	21	Ferulic acid	82	0.04–3.74	0.54	0.29	0.45–4.97
	23	Isoferulic acid	80	0.05–1.41	0.44	0.26	0.58–4.89
	24	Sinapic acid	12	0.08–0.51	0.31	0.36	1.15–4.38
	20	*m*-Coumaric acid	0				
	25	*o*-Coumaric acid	0				
Phenylacetic acid hydroxyderivatives	8	4-Hydroxyphenylacetic acid	84	0.07–1.53	0.40	0.16	0.64–4.76
	9	3-Hydroxyphenylacetic acid	22	0.11–0.12	0.12	0.12	1.08–4.94
	10	2-Hydroxyphenylacetic acid	0				
	4	Homoprotocatechuic acid	22	0.06–0.32	0.15	0.11	1.24–4.87
Sensorically significant compounds	15	Vanillin	60	0.12–54.8	9.85	3.17	0.50–4.76
	22	Ethylvanillin	42	0.050–31.0	2.47	0.47	0.83–4.55
Other phenolic compounds	6	Protocatechuicaldehyde	64	0.03–0.12	0.07	0.05	2.30–4.08
	12	Esculetin	20	0.12	0.12	0.12	4.65
	14	(+)-Catechin	10	0.44–4.04	1.22	0.58	0.72–3.86
	16	Isovanillin	0				

[a] Content > LOD.

the samples of the almond meads (samples no. 40 and 42). For the chromatogram of the sample no. 42 see Fig. 6.

The group of an uncategorized compounds called as other phenolic compounds is formed by protocatechuicaldehyde, esculetin, (+)-catechin and isovanillin. The most occurred compound was protocatechuicaldehyde (64%) but only at very low concentrations (at maximum 0.12 mg/L, sample no. 34). Esculetin was detected in 20% meads, but the concentration above the limit of quantification was determined in one blueberry mead only (0.12 mg/L, sample no. 28). (+)-Catechin was detected in 10% of meads from two manufacturers and the highest amount was determined in the mead with added sour cherry juice (4.04 mg/L, sample no. 33). Isovanillin comes from propolis, but it was not detected in any sample.

3.5. Analysis of hydroxymethylfurfural

The confirmation of hydroxymethylfurfural peak in mead samples was carried out by the comparison of retention time (7.8 min) before and after spiking of a mead sample with the standard compound measured at the same conditions. The quantitative analysis of hydroxymethylfurfural was performed by the linear calibration curve (peak area vs. concentration) including nine calibration points. Parameters of the calibration equation: concentration range 0.5–18 mg/L, slope 50.28 ± 0.44 (mAU L/mg), intercept -2.07 ± 3.28 (mAU), correlation coefficient 0.9996 and RSD range 0.27–0.85%. The limit of detection (0.05 mg/L) was expressed as the signal three times of the baseline noise and the limit of quantification (0.17 mg/L) as the signal 10 times of the baseline noise.

Hydroxymethylfurfural was determined in each mead sample in a wide concentration range 2.47–158 mg/L (average 37.3 mg/L, median 26.5 mg/L). Hydroxymethylfurfural concentration below 10 mg/L was found out in 26% of meads, 10–100 mg/L in 64% of meads and higher concentrations (over 100 mg/L) in 10% of meads. All complete results are listed in Table 4 (parts 1–5).

Fresh honey contains approx. 1–5 mg/L of hydroxymethylfurfural. The maximum limit of hydroxymethylfurfural concentration in honey is defined by the Codex Alimentarius (FAO/WHO Food Standards, Codex Standard for Honey – last revision 2001) on 40 mg/kg. In case of meads made without heat treatments during the mead production from the weight proportion of honey and water in the common range (0.5:1–1:3), maximal hydroxymethylfurfural concentration should be in the range 10–26.4 mg/L. On the basis of this presumption, it is possible to establish that meads with hydroxymethylfurfural concentration higher than 26.4 mg/L (50% mead samples) were made from low-class honey or with any heat treatments during mead production. This presumption does not include mead ageing; further factors should not influence hydroxymethylfural concentration.

4. Conclusions

The HPLC method with coulometric-array detection provides the selectivity and sensitivity well suited to the analysis of many electroactive compounds in complex nature matrixes without any sample pre-treatment. In 50 mead samples obtained from the Czech trade network 19 phenolic compounds from 25 monitored compounds were determined and quantified. Phenolic compounds profile of meads is strongly influenced by mead composition (mainly by fruit juices addition), but it is not easy to find clear markers related to particular modifier. Hydroxymethylfurfural was determined in each mead sample at a wide concentration range and 10% of mead samples contained over 100 mg/L confirming improper processing or storage of meads. The influence of heat treatment and the way of storage on the phenolic compounds and hydroxymethylfurfural concentration in mead produced without any heat treatment during the production is being further studied.

Acknowledgments

This work was supported by the grant project No. MSM002162 7502 sponsored by the Ministry of Education, Youth and Sports of the Czech Republic and project 203/08/1536 sponsored by the Czech Science Foundation. Authors thank to Dr. Karel Bojda and Mr. Evžen Báchor for valuable advices and mead samples.

References

[1] A. Přidal, Včelí produkty, Mendelova zemědělsá a lesnická univerzita v Brně, 2003.
[2] R. Sawicka, Prace Instytutów i Laboratoriów Badawczych Przemyslu Spożywczego, 20 (1970) 411.
[3] W. Wzorek, E. Konieczna, K. Szymańska, A. Bugajevska, Pol. J. Food Nutr. Sci. 2 (1983) 57.
[4] R.W. Kime, M.R. McLellan, C.Y. Lee, Am. Bee J. 131 (1991) 394.
[5] M.N. Pons, S. Schutze, J. Ferment. Bioeng. 78 (1994) 450.
[6] B. Bahiru, T. Mehari, M. Ashenafi, Food Microbiol. 23 (2006) 277.
[7] C.L. Wintersteen, L.M. Andrae, N.J. Engeseth, J. Food Sci. 70 (2005) C119.
[8] P. Sroka, T. Tuszyński, Food Chem. 104 (2007) 1250.
[9] S. Preys, G. Mazerolles, P. Courcoux, A. Samson, U. Fischer, M. Hanafi, D. Bertrand, V. Cheynier, Anal. Chim. Acta 563 (2006) 126.
[10] S. Kreutzmann, L.P. Christensen, M. Edelenbos, LWT-Food Sci. Technol. 41 (2008) 193.
[11] N. Paixão, R. Perestrelo, J.C. Marques, J.S. Câmara, Food Chem. 105 (2007) 204.
[12] D. Matějíček, O. Mikeš, B. Klejdus, D. Štěrbová, V. Kubáň, Food Chem. 90 (2005) 791.
[13] M. Luther, J. Parry, J. Moore, J. Meng, Y. Zhang, Z. Cheng, L. Yu, Food Chem. 104 (2007) 1065.
[14] E.A. Tosi, E. Ré, M.E. Ortega, A.F. Cazzoli, Food Chem. 104 (2007) 1025.
[15] B. Halliwell, Cardiovasc. Res. 73 (2007) 341.
[16] E. Küpeli, F.P. Şahin, İ. Çalış, E. Yeşilada, N. Ezer, J. Ethnopharmacol. 112 (2007) 356.
[17] M.J. Rodríguez Vaquero, M.R. Alberto, M.C. Manca de Nadra, Food Control 18 (2007) 93.
[18] A. King, G. Young, J. Am. Diet. Assoc. 99 (1999) 213.
[19] C. Gerhäuser, Eur. J. Cancer 41 (2005) 1941.
[20] E. Steeg, A. Montag, Z. Lebensm, Unters. Forsch. 187 (1988) 115.
[21] E. Jörg, G. Sontag, D. Lebens, Rundsch 88 (1992) 179.
[22] F. Ferreres, P. Andrade, F.A. Tomás-Barberán, J. Agric. Food Chem. 44 (1996) 2053.
[23] F. Ferreres, T. Juan, C. Pérez-Arquillué, A. Herrera-Marteache, C. García-Viguera, F.A. Tomás-Barberán, J. Sci. Food Agric. 77 (1998) 506.
[24] F.A. Tomás-Barberán, I. Martos, F. Ferreres, B.S. Radovic, E. Anklam, J. Sci. Food Agric. 81 (2001) 485.
[25] B. Dimitrova, R. Gevrenova, E. Anklam, Phytochem. Anal. 18 (2007) 24.
[26] G. Achilli, G.P. Cellerino, P.H. Gamache, J. Chromatogr. 632 (1993) 111.
[27] P. Jandera, V. Škeříková, L. Řehová, T. Hájek, L. Baldriánová, G. Škopová, V. Kellner, A. Horna, J. Sep. Sci. 28 (2005) 1005.
[28] P. Gamache, E. Ryan, I.N. Acworth, J. Chromatogr. 635 (1993) 143.
[29] L. Montanari, G. Perretti, F. Natella, A. Guidi, P. Fantozzi, Lebens. Wissensch. Technol. 32 (1999) 535.
[30] M. Nardini, A. Ghiselli, Food Chem. 84 (2004) 137.
[31] L. Řehová, V. Škeříková, P. Jandera, J. Sep. Sci. 27 (2004) 1345.
[32] T. Nurmi, S. Heinonen, W. Mazur, T. Deyama, S. Nishibe, H. Adlercreutz, Food Chem. 83 (2003) 303.
[33] G. Shui, L.P. Leong, J. Chromatogr. A 977 (2002) 89.
[34] A.A. García, B.C. Grande, J.S. Gándara, J. Chromatogr. A (2004) 175.
[35] Y.T. Lin, D. Vattem, R.G. Labbe, K. Shetty, Process Biochem. 40 (2005) 2059.
[36] N.E. Es-Safi, V. Cheynier, M. Moutounet, J. Food. Comp. Anal. 16 (2003) 535.
[37] L. Bravo, L. Goya, E. Lecumberri, Food. Res. Int. 40 (2007) 393.