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Determination of Free Phenolic Acids in Wort and Beer by Coulometric Array Detection

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The simultaneous determination of 19 phenolic compounds was performed directly in wort and beer by a combination of reverse-phase high-performance liquid chromatography coupled with coulometric array detection. Chromatographic separation was achieved with an appropriate gradient of flow and a binary solvent based on phosphate buffer, methanol, and acetonitrile in a 45-min run. Eight serial coulometric detectors were used for on-line generation of voltammetric data to resolve coeluting compounds. The method was reliable and sensitive, the regression coefficient of standard calibration curves is $0.972 \leq r \leq 1.000$, and the standard deviation value ranges from 0.010 to 0.129 mg/L for wort and from 0.002 to 0.332 mg/L for beer. The mean concentrations of phenolic acids were 22.1 and 33.8 mg/L, respectively, in worts and beers produced in Italy. These amounts represent 5 and 10% of the non-tannic, non-flavonoid phenols in wort and beer, respectively.

KEYWORDS: Phenolic acids; beer; wort; coulometric detection; HPLC-ECD

INTRODUCTION

Phenolic compounds are important components of many fruits, vegetables, and beverages, to which they contribute to flavor, color, and sensory properties such as bitterness and astringency. The presence of phenolics in food may have an important effect on the oxidative stability and microbial safety of products (*1*); in addition, many phenolics possess important biological activity related to their inhibitory effects on mutagenesis and carcinogenesis (*2*−*7*). Phenolic compounds may act as antioxidants in the human body, for example, as protective agents against oxidation of ascorbic acid and unsaturated fatty acids (*3*). Recent interest in the use of phenolic compounds in functional foods and medicinal applications has also stimulated interest in their analyses. The phenolic compounds present in food show considerable diversity in their structure and are divided into different classes. Flavonoids and related phenolic compounds exist in a multiplicity of complex conjugates with esters, sugars, and organic acids. Phenolic acids are among the classes of simple monocyclic acids. Phenolic acids embrace the hydroxy derivates of the benzoic (C_6-C_1) and cinnamic acids (C_6-C_3) (*8*), as reported in **Figure 1**.

Beer contains many phenols, the greater part of which comes from the malt, the remaining portion from the hops (*9*). Phenolic acids can be found in germinated barley as free form or bound to the cell walls. They contribute to the antioxidant activity in beer (*10*). Phenols in beer are present in both monomeric and polymeric forms. Monomers are phenolic acids, flavonols, and their glucosides: catechins, anthocyanogens, and coumarins (*11*).

Studies on the composition of and analytical procedures for determining phenolic acids in wort and beer are limited. Only a few phenolic acids and related compounds have been identified by high-performance liquid chromatography (HPLC) coupled with an electrochemical detector (ECD) or other detectors (*12*−*17*).

Buffer-based HPLC is often used to maintain consistent retention and selectivity. Moreover, a buffered mobile phase resists changes in pH, providing reproducible chromatography. Buffer-based HPLC coupled with ECD allows for the identification of many phenolic acids and other related compounds (i.e., flavonoids, tannins, and catechins) (*18*−*20*). The multichannel coulometric detection system serves as a highly sensitive tool for the characterization of antioxidants because of their electroactivity. The coulometric efficiency of each element of the array allows a complete voltammetric resolution of analytes as a function of their oxidation potential. Some of the peaks may be resolved by the detector even if they coelute.

Recently, Montanari et al. (*21*) determined 16 phenolic acids in beer by coulometric array detection using a binary gradient of 0.1 M phosphate buffer.

The aim of the present work was to characterize wort and beer phenolic acids quantitatively and qualitatively in order to monitor the effects of technologies in the brewing process. The chromatographic method of Montanari et al. (*21*) was adopted with some modifications. Nineteen different hydroxy derivates of cinnamic, benzoic, and phenylacetic acids and tyrosol in a

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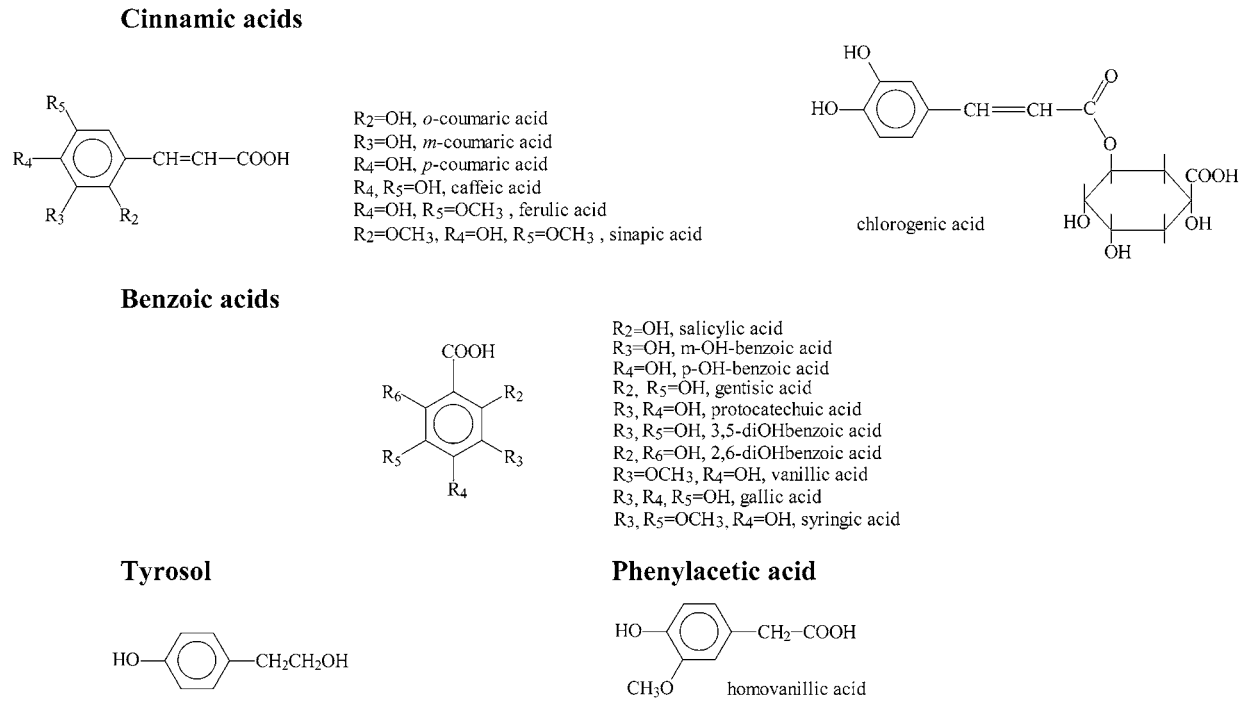

Figure 1. Chemical structures of standards.

45-min run were investigated with a binary gradient of 0.05 M phosphate buffer and 0.05 μM sodium lauryl sulfate (SLS). These compounds were chosen by taking into account the literature values of wort and beer production, technologies, and analyses (*14*, *22*−*24*).

MATERIALS AND METHODS

Chemicals. Gallic acid, 3,5-dihydroxybenzoic acid, gentisic acid, 4-hydroxybenzoic acid, tyrosol, 2,6-dihydroxybenzoic acid, vanillic acid, 3-hydroxybenzoic acid, syringic acid, *p*-coumaric acid, ferulic acid, *m*-coumaric acid, and *o*-coumaric acid were purchased from Fluka (Buchs SG, Switzerland). Protocatechuic acid, homovanillic acid, and sinapic acid were purchased from Carl Roth GmbH and Co. (Karlsruhe, Germany). Chlorogenic acid and caffeic acid were purchased from Sigma (St. Louis, MO). Salicylic acid, methanol (HPLC grade), acetonitrile (HPLC grade), water (HPLC grade), SLS (RS grade), orthophoshoric acid 85% (RPE grade), and potassium monobasic phosphate (RP-ACS grade) were purchased from Carlo Erba (Milano, Italy).

Apparatus. The following equipment was utilized for the HPLC analysis: two Jasco PU-1580 pumps connected to a gradient solvent system, a Basic Marathon "Spark" (Erkerode, The Netherlands) autosampler with a 100-μL loop, an Inertsil ODS-3V (C_{18} 250 mm × 4.6 mm i.d.; particle size = 5 μm) insulated column, a CoulArray (ESA, Inc., Chelmsford, MA) detector, consisting of two cell packs in series, each pack containing four porous graphite working electrode channels with associated palladium reference electrode and platinum counter electrode, and CoulArray Software for Windows for acquisition and elaboration of data.

A dual-beam spectrophotometer (UV−vis) Varian DMS 200 (Varian, Torino, Italy) with 1-cm quartz cells and capable of measuring absorbance in the range of 190−800 nm was used to determine the absorbance for phenolic compounds.

Chromatographic Methods. The composition of the two phases A and B was changed with respect to the chromatographic method of Montanari et al. (*21*). Concentrations of phosphate buffer and SLS were lowered to avoid problems caused by precipitation of buffer in the coulometric cells. A better solvent gradient associated with an appropriate flow rate was also developed. Mobile phase A was 0.05 M KH_2PO_4 and 0.05 μM SLS, and mobile phase B was phase A/CH_3-OH/CH_3CN, 30:20:50 v/v/v, 0.05 μM SLS. The mobile phases were adjusted to pH 3.15 with 85% orthophosphoric acid and were filtered with a 0.22 μm membrane filter (Millipore, Bedford, MA, for aqueous solvents; MSI, MA, for organic solvents). **Table 1** shows the solvent gradient and flow rate during analysis. It represents the same frame of the program in the pump. The variation of flow rate and gradient solvent were linear. The eight electrode potentials were 100−905 mV at increments of 115 mV versus palladium reference electrodes.

Table 1. Solvent Gradient and Flow Rate during Analysis[a]

step	time	A%	flow (ml/min)
initial	*0.0′*	*85*	*0.9*
1	5.0′	80	const.
2	8.0′	80	
3	9.0′	incr.	0.9
4	9.1′		0.4
5	15.0′	85	incr.
6	25.0′	85	
7	26.1′		0.9
8	26.5′		0.9
9	39.4′	decreasing	0.5
10	39.5′		0.5
11	44.0′		0.9
12	44.1′		
13	45.0′	50	constant
14	46.1′	0	
15	53.0′	0	
16	55.0′	85	
final	*60.0′*	*85*	*0.9*

[a] The increase and decrease of the solvent gradient and flow rate were linear.

Standard and Sample Preparation. The stock standard solutions were prepared by dissolving 20−30 mg of each compound in 100 mL of mobile phase A. The stock standard solutions were stored at −4 °C for a maximum of 1 month. The 19 phenolic compound standard solutions were prepared by combining and diluting the individual stock standard solutions to obtain the desired concentrations in the range of 1−3 mg/L for each acid. The working standard mixture was diluted in the ratios 1:2, 1:4, and 1:5 (v/v) to obtain the calibration solutions.

Lager-type beers and worts were provided by Italian Brewing Factories. Beer samples were degassed by sonication, and wort was passed through a 0.22-μm membrane syringe filter (Whatman Inc.,

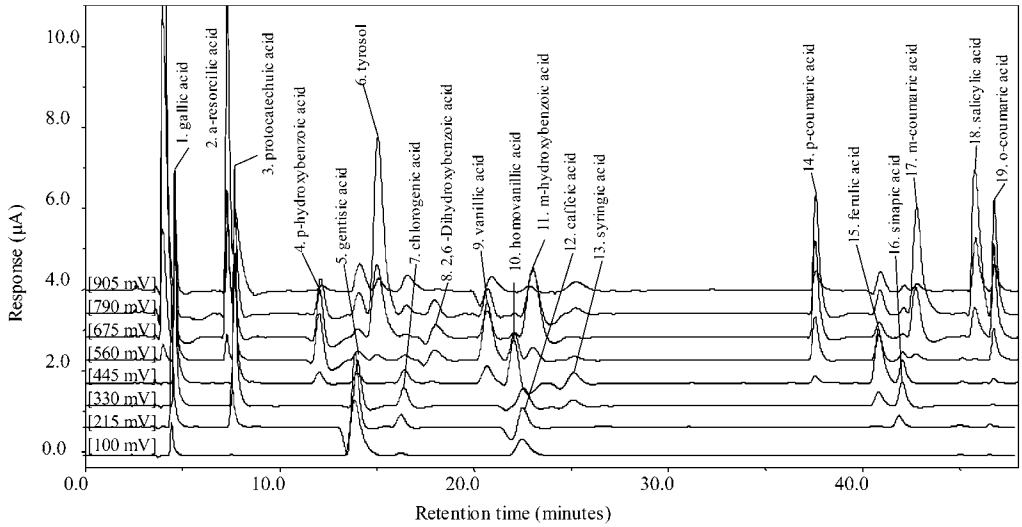

Figure 2. Eight-channel chromatogram of 19-component standard. Cell potential was set from 100 to 905 mV at 115 mV increments. See **Table 1** for compound listing, retention times, and dominant oxidation potential.

Swedesboro, NJ). All of the samples were diluted 2:5 (v/v) with phase A to buffer them at the same pH of the mobile phase.

Data Elaboration. The voltammetric data were collected and analyzed by CoulArray software. A specific retention time and a typical electrochemical response characterize each analyte across the array. The majority of responses for a single oxidation wave typically occurred through three adjacent sensors. The response of analyte across the channels is characteristic and not dependent on its concentration. The highest intensity responding sensor defines the dominant channel, whereas the leading and following channels maintain the same response ratio for each analyte (*16*).

Chemical Analysis. The determination of total, non-tannic, and non-flavonoid phenols was conducted in degassed beer and wort diluted with water in a 1:2 ratio (v/v).

Total Phenols (TP). The content of total phenols in wort and beer was quantified by using the Folin−Ciocalteu assay (*26*): 0.1 mL of degassed beer or 0.4 mL of diluted wort was mixed in a 20 mL test tube with 2 mL of water, 10 mL of Folin−Ciocalteu reagent, and 8 mL of a 7.5% aqueous solution of Na_2CO_3; the solution was heated at 45 °C for 15 min in a thermostatic bath and then cooled to room temperature. At least three determinations were conducted for every analysis. The absorbance (A_{765nm}) of the solutions was determined at 765 nm.

Concentration of total phenols was calculated by the following equation as milligrams per liter of gallic acid and reported as

$$\text{mg/L (gallic acid)} = \frac{A_{765nm}}{0.001443} \times 2$$

Non-tannic Phenols (NTP). The concentration of non-tannic phenols was evaluated after selective precipitation of tannic phenols by methylcellulose (*27*). Five milliliters of diluted wort or degassed beer was mixed with 1 mL of 0.4% aqueous solution of methylcellulose, 2 mL of a saturated aqueous solution of ammonium sulfate, and 2 mL of distilled water. The solution was centrifuged at 3000 rpm for 15 min; 0.2 mL of supernatant for beer or 1 mL for wort was used to determine non-tannic phenols with the Folin−Ciocalteu assay as for total phenols and using the same equation for the calculation.

Non-flavonoid Phenols (NFP). The concentration of non-flavonoid phenols was evaluated after selective precipitation of flavonoid phenols by formaldehyde (*28*). Five milliliters of diluted wort or degassed beer was mixed with 5 mL of 1:4 (v/v) concentrated HCl and 2.5 mL of formaldehyde solution. The solution was stored at room temperature for 24 h and then filtered with a 0.4-μm membrane syringe filter; 0.2 mL of filtered solution for beer or 1 mL for wort was used to determine non-flavonoid phenols with the Folin−Ciocalteu assay.

The content of non-flavonoid phenols was calculated with the following equation and reported as milligrams per liter of gallic acid:

$$\text{mg/L (gallic acid)} = \frac{A_{765nm}}{0.001443} \times \frac{12}{5}$$

RESULTS AND DISCUSSION

The coulometric detector is suitable for the analysis of phenolic compounds because they are electroactive substances that usually oxidize at low potential in beer (*14*, *19*). The extraction of phenolic compounds from wort and beer matrices is not needed because the other compounds, such as fermentable sugars, dextrins, and organic acids, do not interfere with chromatographic response.

Figure 2 shows the chromatogram of all 19 standard compounds. In these conditions, the ratio of methanol and acetonitrile was increased in phase B with respect to the method of Montanari et al. (*21*). In general, increasing the methanol concentration decreases the retention time of phenolic compounds. In the first three steps of the gradient, a lower percentage of phase B led to a better separation between 3,5-dihydroxybenzoic and protocatechuic acids (peaks 2 and 3). Increasing the methanol concentration decreased the retention time of cinnamic acid derivatives more than that of benzoic acid derivatives. This is due to cinnamic acid's better solubility in methanol (*14*). Consequently, the peaks of caffeic, syringic, and vanillic acids began to overlap. The flow rate gradient and the increase of phase A were essential to the separation of these peaks (0.4 mL/min, 85% phase A) allowing a good separation for homovanillic, caffeic, 3-hydroxybenzoic, and syringic acids (peaks 9−13). The progressive increase of phase B from 15% (step 5, 25 min) to 50% (step 13, 45 min) reduced the retention time of the cinnamic acids, salicylic acid (peaks 14−19), and the total analysis time. A flow rate of 0.5 mL/min was needed to separate ferulic and sinapic acids. The starting system conditions were restored at the end of the chromatographic separation; in particular 7 min of 100% phase B allowed the elution of all residual compounds from the column.

The retention times (RT) of 19 standard compounds are reported in **Table 2**. The elution of phenolic compounds follows the decreasing polarity in reversed-phase HPLC so benzoic acid derivates are eluted earlier than cinnamic acid derivates. Guo et al. (*19*) reported that the retention time of phenolic compounds increases with the number of $-OCH_3$ substituents. Gallic acid is the first acid eluted (three −OH groups), whereas vanillic acid, the first $-OCH_3$ substituted among benzoic acids, has an

Table 2. Regression Coefficient of Peak Areas versus Concentration (Milligrams per Liter) of the Standard Compounds

peak	IUPAC name	current name	RT[a] (min)	DP[b] (mV)	slope	intercept	r^c
1	3,4,5-trihydroxybenzoic acid	gallic acid	4.58	445	42.370	0.090	0.980
2	3,5-dihydroxybenzoic acid	α-resorcylic acid	7.28	790	146.340	0.023	0.996
3	3,4-dihydroxybenzoic acid	protocatechuic acid	7.67	330	55.680	–0.065	0.999
4	4-hydroxybenzoic acid	*p*-hydroxybenzoic acid	12.07	560	53.810	–0.018	0.994
5	2,5-dihydroxybenzoic acid	gentisic acid	14.03	100	62.385	–0.075	0.996
6	2-(4-hydroxyphenyl)ethyl alcohol	tyrosol	15.05	790	87.956	–0.264	0.991
7	3-(3,4-dihydroxycinnamoyl)quinic acid	chlorogenic acid	16.39	445	17.999	–0.024	0.994
8	2,6-dihydroxybenzoic acid		18.03	675	34.631	–0.023	0.998
9	4-hydroxy-3-methoxybenzoic acid	vanillic acid	20.68	560	35.364	–0.994	0.991
10	4-hydroxy-3-methoxyphenylacetic acid	homovanillic acid	22.05	445	32.553	0.065	0.991
11	3,4-dihydroxycinnamic acid	caffeic acid	22.65	215	21.209	–0.006	0.976
12	3-hydroxybenzoic acid	*m*-hydroxybenzoic acid	23.02	790	34.811	0.001	0.990
13	3,5-dimethoxy-4-hydroxybenzoic acid	syringic acid	25.13	445	20.296	0.000	1.000
14	*trans*-4-hydroxycinnamic acid	*p*-coumaric acid	37.60	790	82.170	0.029	0.995
15	4-hydroxy-3-methoxycinnamic acid	ferulic acid	40.83	445	36.696	–0.040	0.997
16	3,5-dihydroxy-4-hydroxycinnamic acid	sinapic acid	42.05	330	23.078	0.033	0.992
17	*trans*-3-hydroxycinnamic acid	*m*-coumaric acid	42.80	790	84.514	0.039	0.977
18	2-hydroxybenzoic acid	salicylic acid	45.78	790	96.999	–0.437	0.972
19	*trans*-2-hydroxycinnamic acid	*o*-coumaric acid	46.80	790	76.7558	0.006	0.998

[a] Retention time. [b] Dominant potential. [c] Correlation coefficient ($P < 0.0001$).

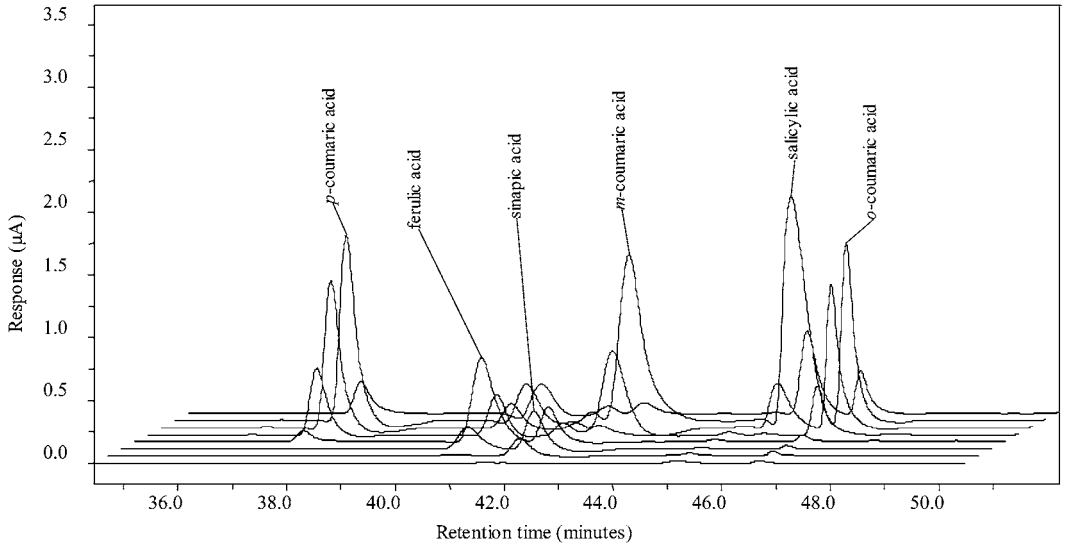

Figure 3. Eight-channel chromatogram of cinnamic group and salicylic of standard solution. Cell potential was set from 100 to 905 mV at 115 mV increments as in **Figure 2**.

RT of 20.68 min. This is confirmed by syringic acid (two $-OCH_3$ substituents), which has an RT of 25.13 min. Salicylic acid is an exception; it has only one $-OH$ substitution and is eluted with the cinnamic acids. This may be the consequence of intermolecular hydrogen bonding (*29*). Chlorogenic acid has the shortest retention time of the cinnamic acids owing to the presence of a sugar moiety that increases its mobility, as found for flavonoid compounds by Guo et al. (*19*).

The reproducibility of the method adopted was tested by injecting a 19 standards solution four times. Each linear regression for peak area (microcoulomb, μC) versus concentration (mg/L) is extrapolated from 16 points. The regression coefficient of peak area versus concentration is reported in **Table 2**. The correlation coefficients (r) of linear regressions were calculated for $P < 0.0001$ and were good for every standard compound tested.

In **Figure 2** each compound is represented in its first oxidation potential; however, peaks 1, 3, 5, 7, 9, 13, 15, and 16 show a secondary oxidation potential in these experimental conditions. Each compound showed a different response by the channel as can be seen in **Figure 3**, which reports a small segment of the standard chromatogram in which with a trained eye combined chromatographic and voltammetric resolution is evident for *p*-coumaric, ferulic, sinapic, *m*-coumaric, salicylic, and *o*-coumaric acids.

ECD resolution is based on differences in relative ease of oxidation and, therefore, on the structural and electronic properties of a molecule. Among cinnamic compounds having a catechol group (caffeic and chlorogenic acids) all responded at low oxidation potentials (*20*). Methoxycatechol analogues responded at higher potentials than the catechols (e.g., sinapic acid, 330 mV; and ferulic acid, 445 mV). Monophenolic coumaric acids oxidized at higher potentials still (~790 mV) with ortho and para isomers responding at slightly lower potentials than *m*-coumaric acid.

The samples for the analysis were injected after a simple preparation. They were only filtered and diluted with solvent A. An accurate study was carried out to determine the correct dilution. Samples must be diluted with solvent A (2:5 v/v) to be buffered at the same pH as the mobile phase to avoid RT changes. In **Figures 4** and **5** are reported typical elution profiles of wort and beer, respectively. In the wort and beer chromato-

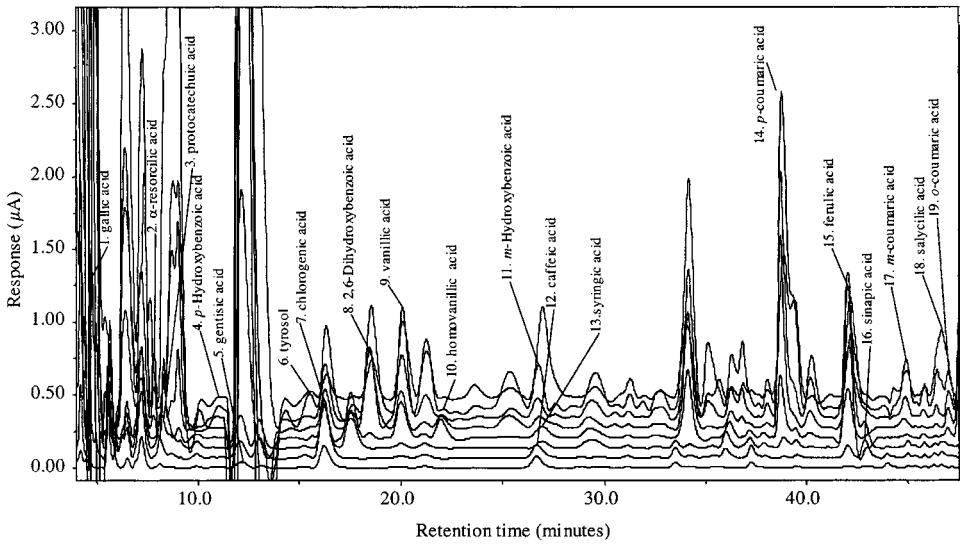

Figure 4. Typical elution profile of wort sample. Cell potential was set from 100 to 905 mV at 115 mV increments as in **Figure 2**. See **Table 2** for single free phenolic acids value determined.

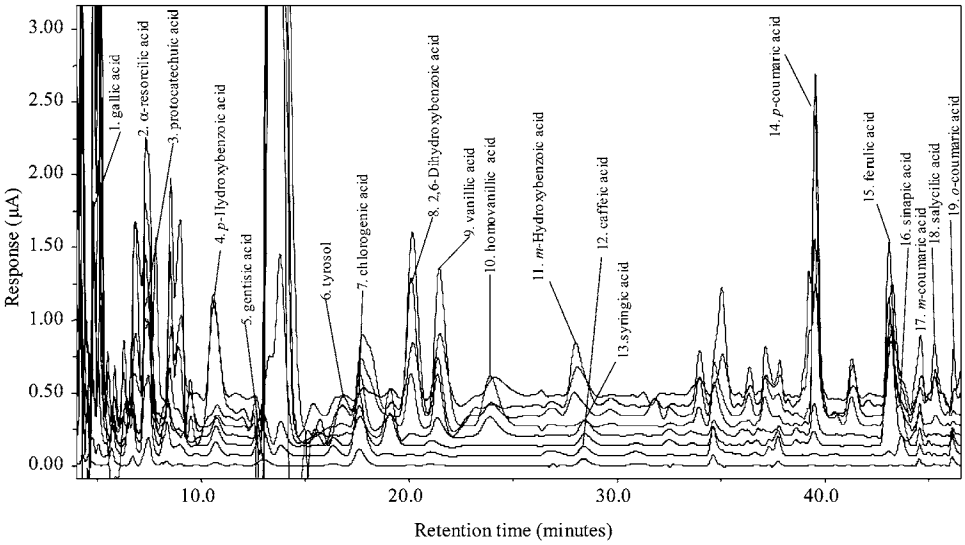

Figure 5. Typical elution profile of beer sample. Cell potential was set from 100 to 905 mV at 115 mV increments as in **Figure 2**. See **Table 2** for single free phenolic acids value determined.

grams, among the 19 compounds detected, other more electroactive compounds are resolved. In particular, in both wort and beer, between 12 and 14 min there is an sizable unknown peak with respect to the compounds resolved and detected.

The concentrations of 19 free phenolic acids (FPA) of wort and beer produced in Italy are reported in **Table 3**. The standard deviation (SD) value ranges from 0.010 to 0.129 mg/L for wort and from 0.002 to 0.332 mg/L for beer. The total amounts of FPA detected are 13.8 and 29.2 mg/L in wort and beer, respectively. These data confirm that the proposed method is reliable and sensitive for all of the selected phenolic compounds.

Table 4 reports the concentration of the 19 phenolic acids, the total amount of FPA as sum of compounds detected, and the values of total phenols (TP), non-tannic phenols (NTP), and non-flavonoid phenols (NFP) in 23 samples of wort and beer. All of the values fit with the data reported in the literature (*13*, *22*, *25*, *30*). The value of each FPA is the mean of 23 different brand samples analyzed in triplicate. The wort and beer samples are not related. Ferulic and salicylic acids are found in major amounts, 3.8 and 5.3 mg/L, respectively, in wort samples. *p*-Hydroxybenzoic acid represents 50% of the total average amount of FPA, determined by HPLC-ECD, in beer samples. The SD value ranges from 0.1 to 4.0 mg/L for wort and from 0.1 to 11.0 mg/L for beer. The different sources of the samples analyzed justify the high value of the SD. The sums of FPA are 22.1 and 33.8 mg/L in wort and beer, respectively. For both wort and beer matrices the SD value of the sum of FPA is lower, in percentage, than the SD values of each phenolic acid. This indicates that the total amounts of FPA analyzed in each sample are similar but that the relative composition changes. The concentrations of TP, NTP, and NFP in worts and beers indicate that the majority of phenolic compounds in wort and beer are non-tannic and non-flavonoid (NTNF), or even phenolic acids, as confirmed by previous studies (*11*, *21*). NTNF are 87% of TP (539 mg/L) in wort and 98% of TP (388 mg/L) in beer.

With this HPLC-ECD method only free phenolic acids were detected, whereas the phenolic acids bonded to other molecules were not detected. In the TP of beer there are phenolic acids, hydroxycoumarins, catechins, leucoanthocyanidins, anthocyanidins, flavonols, flavonones, flavones, and phenolic glycosides.

Generally, monomeric phenolics account for 10−20% of the total content of beer phenolic compounds (*25*). From **Table 4**,

Table 3. Free Phenolic Acids (FPA) Concentrations by HPLC-ECD in Wort and Beer[a] Produced in Italy

	wort		beer	
compound	mg/L	SD[b]	mg/L	SD[b]
gallic acid	0.315	0.008	0.498	0.034
α-resorcylic acid	0.048	0.009	0.012	0.002
protocatechuic acid	0.379	0.006	0.468	0.013
p-hydroxybenzoic acid	0.348	0.001	9.038	0.332
gentisic acid	0.316	0.017	0.301	0.004
tyrosol	0.430	0.013	1.894	0.201
chlorogenic acid	0.858	0.070	0.928	0.041
2,6-dihydroxybenzoic acid	1.098	0.038	2.526	0.112
vanillic acid	0.645	0.003	1.200	0.132
homovanillic acid	0.525	0.041	0.411	0.043
caffeic acid	0.587	0.017	0.517	0.026
m-hydroxybenzoic acid	0.778	0.048	0.109	0.017
syringic acid	0.383	0.024	0.272	0.016
p-coumaric acid	2.083	0.129	1.304	0.113
ferulic acid	1.916	0.048	2.321	0.080
sinapic acid	0.068	0.002	0.212	0.005
m-coumaric acid	0.084	0.002	0.105	0.006
salicylic acid	2.808	0.021	6.663	0.233
o-coumaric acid	0.097	0.013	0.469	0.036
total FPA	13.8	0.5	29.2	1.5

[a] Data expressed as mean ($n = 3$). [b] Standard deviation.

Table 4. Concentration of Free Phenolic Acids (FPA) by HPLC-ECD in Wort and Beer Produced in Italy

	wort		beer	
compound	mg/L[a]	SD[b]	mg/L[a]	SD[b]
gallic acid	0.703	0.702	0.593	0.631
α-resorcylic acid	0.207	0.309	0.348	0.601
protocatechuic acid	0.420	0.158	0.840	1.663
p-hydroxybenzoic acid	1.642	1.419	16.840	10.988
gentisic acid	0.328	0.253	0.376	0.307
tyrosol	1.774	1.656	2.906	5.947
chlorogenic acid	1.354	0.547	0.901	0.489
2,6-dihydroxybenzoic acid	1.383	0.655	0.916	0.462
vanillic acid	0.643	0.194	0.737	0.251
homovanillic acid	0.482	0.133	0.580	0.112
caffeic acid	0.563	0.163	0.566	0.336
m-hydroxybenzoic acid	0.686	0.506	0.324	0.212
syringic acid	0.209	0.141	0.237	0.096
p-coumaric acid	1.963	0.824	1.364	0.709
ferulic acid	3.782	2.015	2.410	0.875
sinapic acid	0.438	1.223	0.151	0.152
m-coumaric acid	0.384	0.209	0.227	0.328
salicylic acid	5.306	3.939	2.866	1.556
o-coumaric acid	0.306	0.237	1.731	3.741
total FPA	22.1	5.4	33.8	13.8
TP[c]	539	127	388	165
NTP[d]	447	108	378	164
NFP[e]	497	127	382	165

[a] Values are the means of 23 different samples. [b] Standard deviation. [c] Total phenols. [d] Non-tannic phenols. [e] Non-flavonoid phenols.

the FPA sums are about 5 and 9% of TP in wort and beer, respectively. The lower percentage value found in beer with respect to the literature is due to the limited number of compounds that were monitored in this study. Probably in the literature data a larger number of FPA are accounted for.

Conclusion. HPLC analysis coupled with an electrochemical detector allows separation of homologous phenolic acids in wort and beer. This HPLC-ECD analysis was set up to routinely analyze up to 19 phenolic compounds in order to control the brewing process and the composition of the final product. The advantage of this procedure is a reproducible result obtained by direct injection of wort and beer without sample preparation. The influence of the brewing process on the content of free phenolic acids of beer can be easily evaluated. Covalently bonded phenolic compounds in beer will be investigated in future studies. A method will be developed for the hydrolysis and extraction for determining the total concentration (free or bound) of phenolic acids, including some other phenolic acids resolved with this method but not determined in this paper.

NOTE ADDED AFTER ASAP POSTING

An incorrect version of Figure 4 was included in the original ASAP posting of January 24, 2003. The correct figure is shown in this posting.

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