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# Effects of Processing Steps on the Phenolic Content and Antioxidant Activity of Beer

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**ABSTRACT:** A new analytical method (liquid chromatography−antioxidant, LC-AOx) was used that is intended to separate beer polyphenols and to determine the potential antioxidant activity of these constituents after they were allowed to react online with a buffered solution of the radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$). Using the LC-AOx method, it was possible to demonstrate that the extent of the antioxidant activity was very much dependent on the phenolic compound considered. The method was also applied to the analysis of beer extracts and allowed the evaluation of their antioxidant activity at different steps of beer processing: brewing, boiling, and fermentation. This study showed that the total antioxidant activity remained unchanged throughout beer processing, as opposed to the polyphenolic content, which showed a 3-fold increase. Hopping and fermentation steps were the main causes of this increase. However, the increase measured after fermentation was attributed to a better extraction of polyphenols due to the presence of ethanol, rather than to a real increase in their content. Moreover, this method allowed the detection of three unknown antioxidant compounds, which accounted for 64 ± 4% of the total antioxidant activity of beer and were individually more efficient than caffeic acid and epicatechin.

**KEYWORDS:** Beer, polyphenol, liquid chromatography, antioxidant activity, ABTS

## INTRODUCTION

Plant-based diets rich in fruits and vegetables are associated with a reduced risk of chronic diseases including coronary heart disease and some cancers.[1] Plant foods contain numerous molecules that, by acting through various mechanisms, provide protection against such diseases. Among these molecules, attention is particularly directed toward phenolic compounds with antioxidant activity. Polyphenols are present not only in fruits and vegetables but also in cereal crops, such as barley and its products, which are the focus of increasing interest due to their high content in phenolic acids (e.g. benzoic and cinnamic acid derivatives), proanthocyanidins, tannins, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds.[2] Barley is among the most ancient and most widely consumed cereal crops, mainly (80−90%) destined to animal feed and malt production.[3] Even after malting, barley seems to retain high amounts of phenolic compounds and an associated antioxidant potential.[4,5]

Phenolic compounds constitute the main class of natural antioxidants present in plant foods and may function as reducing agents, free radical scavengers, singlet oxygen quenchers, and potential complexers of prooxidants. They also confer protection against biological macromolecular damage, significantly prevent the decrease of antioxidant enzyme activity in the aging brain and liver, decrease brain and liver malondialdehyde level and carbonyl content, and improve the total antioxidant capability in the organism.[4]

Phenolic and polyphenolic compounds are usually investigated by liquid chromatography coupled with detection by absorption photometry, due to the presence of chromophores in their structures. Although this allows a fairly good quantitative assessment of the compounds, when chemical standards are available, the antioxidant activity of the analyzed compounds, which varies as a function of their chemical structures, is often overlooked. Given that not all polyphenols present an antioxidant activity and that some are more active than others,[6] inconsistencies regarding the actual interest of many polyphenols and their nutritional allegations are widespread in scientific documents. Furthermore, studies of the antioxidant activity of natural extracts show that known antioxidants account for only a fraction of the total activity and that a large part of biologically important compounds is still unknown.[7] This shows the need for a more pragmatic alternative that would focus on detecting and quantitating the biological activity, rather than the molecule itself.

In the past 10 years, several sensitive postcolumn HPLC methods (liquid chromatography−antioxidant, LC-AOx) for the analysis of the antioxidant activity have been published.[8−10] One of these methods requires a stable model free radical system, such as the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$), the absorbance of which at 734 nm decreases upon reaction with a reducing agent.[8,11] This reaction is associated with the ability of the molecule of interest to trap radicals and in turn with its biological activity. The radical scavenging activity is generally assessed against a standard antioxidant, such as the water-soluble synthetic vitamin E derivative 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox).[12,13] This online

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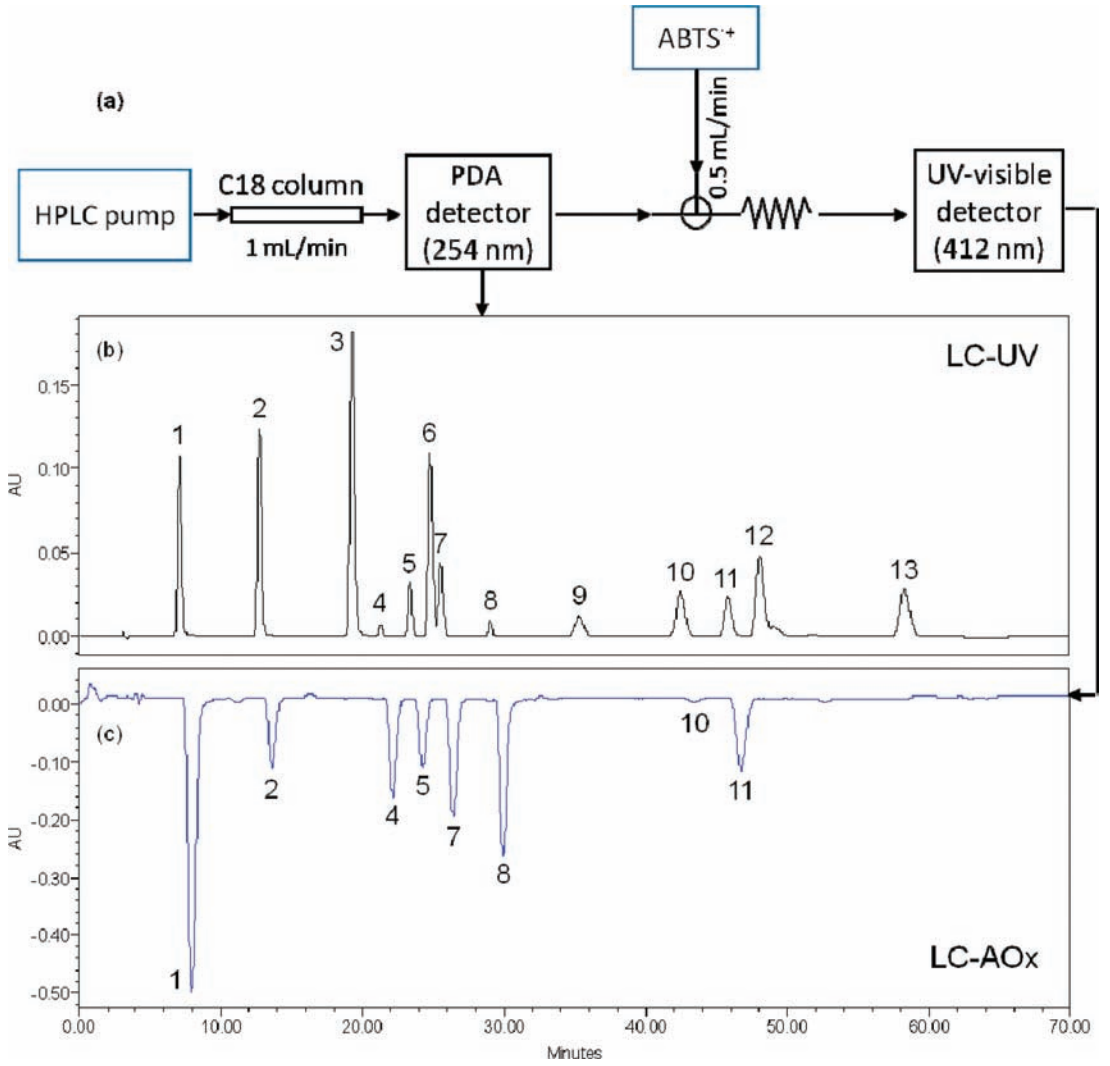


**Figure 1.** Chromatographic separation and detection of phenolic compounds: (a) experimental chart; (b) polyphenol standards at 30 $\mu$g/mL in methanol/water (50:50, v/v); (c) their corresponding antioxidant activity. Peaks: 1, gallic acid; 2, protocatechuic acid; 3, *p*-hydroxybenzoic acid; 4, catechin; 5, chlorogenic acid; 6, vanillic acid; 7, caffeic acid; 8, epicatechin; 9, *p*-coumaric acid; 10, ferulic acid; 11, sinapic acid; 12, *m*-coumaric acid; 13, *o*-coumaric acid.

assessment of antioxidant activity allows complex mixtures to be separated by HPLC and the antioxidant contribution of individual components to be separately evaluated.[12] In addition, the detection of unknown molecules with antioxidant activities in such mixtures will be made much easier.

In the present study, ethyl acetate extracts of beer at different stages of its processing were analyzed for their content in antioxidant compounds using an HPLC system linked to an $ABTS^{\bullet+}$-based postcolumn antioxidant detection system (LC-AOx). Zhao et al. showed that the brewing process might have a considerable impact on the ABTS radical cation scavenging activity of beer.[14] The purpose was to directly investigate the effect of the various processing steps on both the content and the antioxidant activity of beer phenolic compounds.

## MATERIALS AND METHODS

**Chemicals and Products.** $ABTS^{\bullet+}$, Trolox, 4-hydroxy-3-methoxycinnamic acid (ferulic acid), 3,4-dihydroxycinnamic acid (caffeic acid), 3,4,5-trihydroxybenzoic acid (gallic acid), 4-hydroxy-3-methoxybenzoic acid (vanillic acid), 3,4-dihydroxybenzoic acid (protocatechuic acid), *p*-hydroxybenzoic acid, chlorogenic acid, 4-hydroxycinnamic acid (*p*-coumaric acid), sinapic acid, *m*-coumaric acid, *o*-coumaric acid, catechin, and epicatechin were purchased from Sigma-Aldrich (Seelze, Germany). All chemicals and solvents used were of HPLC grade and were purchased from VWR (Strasbourg, France). Ultrapure water was produced by a Synergy UV purification system (Millipore, Molsheim, France). Barley was of the Sunshine variety and was malted, brewed, and fermented by Brasseries Kronenbourg (Strasbourg, France). Hop was purchased from Yakima Chief, Inc. (Sunnyside, WA) in the form of a resinous phase of α-acids, $\beta$-acids, oils, and uncharacterized resins produced by $CO_2$ supercritical extraction and used as a bittering extract added at the final brewing step.

**Stock and Working Solutions.** A stable stock solution of $ABTS^{\bullet+}$ was produced by mixing a 7 mmol $L^{-1}$ aqueous solution of ABTS with a 2.5 mmol $L^{-1}$ solution of potassium persulfate (final concentration) and allowing the mixture to stand in the dark at 4 °C overnight.[8,9] Before use, an $ABTS^{\bullet+}$ working solution was obtained by diluting the stock solution in ethanol to reach an absorbance of 0.70 (±0.02) AU at 734 nm. Stock solutions of phenolic standards were prepared by dissolving the compounds in methanol (1 mg/mL) and were then stored in the dark at −20 °C. Before use, working solutions were prepared by diluting the stock solutions in methanol/water (50:50, v/v).

**Table 1. Quantification Limits of Some Phenolic Compounds in Methanol/Water (50:50, v/v) for the LC-UV and the LC-AOx Detection Methods**

| | quantification limit (pmol) | |
|---|---|---|
| phenolic compound | LC-UV | LC-AOx |
| gallic acid | 94 | 59 |
| protocatechuic acid | 10 | 779 |
| *p*-hydroxybenzoic acid | 29 | -[a] |
| catechin | 54 | 103 |
| chlorogenic acid | 2 | 141 |
| vanillic acid | 15 | − |
| caffeic acid | 4 | 167 |
| epicatechin | 541 | 54 |
| *p*-coumaric acid | 190 | − |
| ferulic acid | 7 | 2575 |
| sinapic acid | 14 | 268 |
| *m*-coumaric acid | 98 | − |
| *o*-coumaric acid | 39 | − |

[a] −, no antioxidant activity.

**Sampling and Extraction at Different Stages of Beer Processing.** Malt was brewed with water to obtain wort (13 °Plato) under the following brewing diagram: 0−20 min, 37 °C; 20−34 min, 37−50 °C; 34−44 min, 50 °C; 44−59 min, 50−65 °C; 59−69 min, 65 °C; 69−82 min, 65−76 °C; 82−92 min, 76 °C. Wort was boiled without hop (1 h, 100 °C) to obtain boiled wort and then was fermented to obtain fermented boiled wort. Wort was boiled with hop (1 h, 100 °C) to obtain boiled hopped wort and then was fermented to obtain beer. Hop used was resinous extracts obtained by $CO_2$ supercritical extraction, and even if it contains no polyphenols, it is the most commonly used ingredient added in wort by industrial brewers.

**Extraction of Phenolic Compounds.** Phenolic compounds were extracted after each previous production step. Sample solutions (10 mL) were first set at pH 2.0 by the addition of HCl (37%), and then 0.5 g of sodium chloride was added. Extraction was carried out in 50 mL Corning centrifuge tubes with 10 mL of ethyl acetate (three times, for periods of 15 min) on a gyratory shaker at 200 rpm.[5,15] The obtained phenolic extract was centrifuged (5000 rpm, 10 min), and supernatant was evaporated to dryness under vacuum (30 °C, 80 mbar). The residue was dissolved in 1 mL of methanol/water (50:50, v/v), membrane-filtered (0.45 μm, Macherey-Nagel, Hoerdt, France), and injected (20 μL) in the chromatographic system.

**Recovery Rates.** The recovery rates of the extraction method were determined by comparison of data obtained from wort extract and data obtained from the same extract with preliminary addition of phenolic standard compounds. These compounds were added to wort at two different concentrations for each compound. The range of values was from 7 to 200 μM. These concentrations were representative of values of each compound obtained in extracts at different processing steps. The average recovery rates were calculated.

**HPLC Analysis.** HPLC coupled with ABTS (LC-AOx) assay was performed by using the method developed by Koleva et al.[8] and Dapkevicius et al.[9] with slight modifications. The block scheme of the instrumental setup is presented in Figure 1a. The HPLC system (Waters, Saint-Quentin-Fallavier, France) consisted of the following: a 616 controller; a 2996 photodiode array detector to UV detection; a 486 tunable absorbance detector to $ABTS^{\bullet+}$ detection; a 717 plus autosampler; and an additional HPLC pump used for the delivery of the $ABTS^{\bullet+}$ solution. The reaction coil used was made of PEEK tubing of 7 m × 0.5 mm i.d. UV detection was carried out at 254 nm. Detection of

**Table 2. Average Recovery Rates of the Studied Compounds in Wort (Recovery Rates from Six Ethyl Acetate Extractions in LC-UV)**

| phenolic compound | recovery rate (%) |
|---|---|
| gallic acid | 93 ± 16 |
| protocatechuic acid | 64 ± 9 |
| *p*-hydroxybenzoic acid | 83 ± 10 |
| catechin | 75 ± 5 |
| chlorogenic acid | 51 ± 6 |
| vanillic acid | 94 ± 7 |
| caffeic acid | 73 ± 5 |
| epicatechin | 49 ± 10 |
| *p*-coumaric acid | 73 ± 10 |
| ferulic acid | 87 ± 5 |
| sinapic acid | 79 ± 16 |
| *m*-coumaric acid | 125 ± 6 |
| *o*-coumaric acid | 88 ± 9 |

$ABTS^{\bullet+}$ reduction was carried out at 412 nm. Separations were carried out at room temperature on a Hypersil BDS C18 HPLC column (5 μm particle size, 250 × 4.6 mm i.d., Fisher Scientific, Illkirch, France). The mobile phase, delivered at 1 mL/min, consisted of a gradient mixture of water containing 0.1% formic acid (eluent A) and methanol (eluent B). The following gradient was used: 0−25 min, 3−25% B; 25−26 min, 25−18% B; 26−29 min, 18% B; 29−47 min, 18−30% B; 47−57 min, 30% B; 57−67 min, 30−65% B; 67−77 min, 65% B. The $ABTS^{\bullet+}$ solution was delivered at 0.5 mL/min.

**Calibration Graphs.** Calibration graphs for each phenolic compound were drawn from data of three replicate injections of 20 μL of standard mixtures obtained by dilution (methanol/water (50:50, v/v)) at various levels of the stock standard solutions. The curves (six data points, $n$ = 3) were linear with $R^2$ values of >0.99. Each phenolic compound was quantified by reference to its appropriate authentic standard for UV detection, whereas the antioxidant potential was calculated as the concentration of Trolox required to produce an equivalent peak area and expressed as Trolox equivalent (μM).

**Quantification Limits.** The quantification limits were estimated following successive dilutions of standards and considering a signal-to-noise ratio of 10 (Table 1). The precision and trueness of the method were determined on the basis of the coefficient of variation and the recovery (found concentration/expected concentration) calculated from three successive injections. Quantification limits were acceptable only if the coefficient of variation was <10% and the recovery about 100 ± 5%.

**Statistical Analysis.** Data were analyzed by ANOVA (at a significance level of 95%) using Statgraphics Plus software. All samples were analyzed in triplicate.

## RESULTS AND DISCUSSION

**Separation and Identification of Antioxidant Products.** HPLC separation of phenolic compounds from complex natural mixtures usually requires a long linear elution gradient. Because beer extracts are rich in phenolic compounds, the separation was first performed on standards to try to separate them with the highest resolution possible. The used gradient, which started from 3% with an increase of 0.88%/min of methanol, did not allow a good separation of vanillic and caffeic acids. The methanol content was then decreased before being increased again toward the end of the separation, which allowed a much better separation of the two compounds at 30 μg/mL each (Figure 1b).

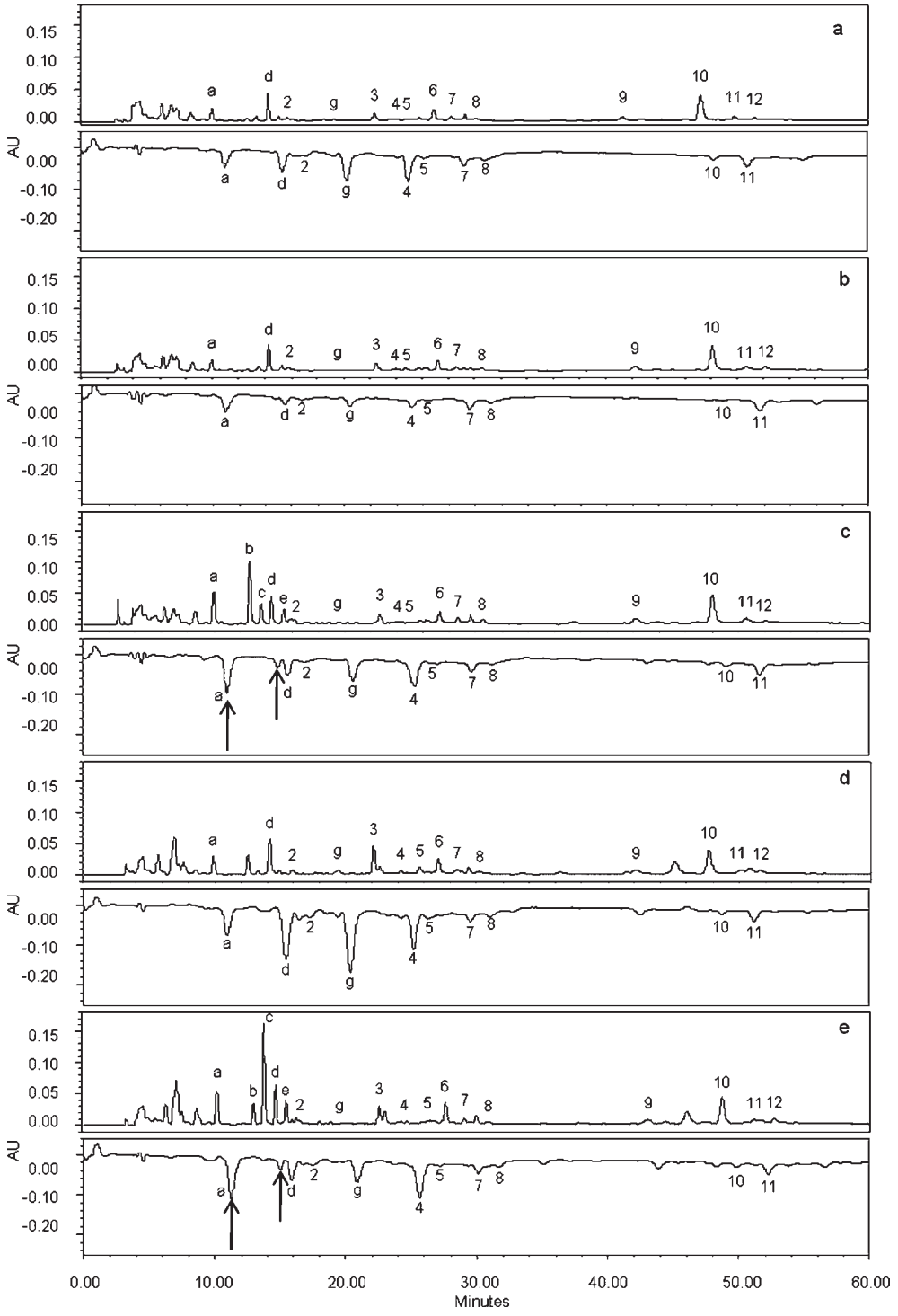


**Figure 2.** Chromatographic determination of antioxidant compounds (upper chromatogram) and their corresponding antioxidant activity (lower chromatogram) in extracts of (a) wort, (b) boiled wort, (c) boiled hopped wort, (d) fermented boiled wort, and (e) beer. Peaks: a−e and g, unknown compounds; 2, protocatechuic acid; 3, *p*-hydroxybenzoic acid; 4, catechin; 5, chlorogenic acid; 6, vanillic acid; 7, caffeic acid; 8, epicatechin; 9, *p*-coumaric acid; 10, ferulic acid; 11, sinapic acid; 12, *m*-coumaric acid.

Conventional methods for identifying antioxidant compounds in complex mixtures typically involve time-consuming assay-guided fractionation procedures, followed by identification of the purified compounds. The system described here (LC-AOx) is meant to screen for compounds with antioxidant activity in natural extracts in a more direct and rapid fashion. Following chromatographic separation, compounds of an extract were mixed online with a stabilized solution of the $ABTS^{\bullet+}$ radical, which was directed to a UV−vis detector. Beekwilder et al.[13] detected $ABTS^{\bullet+}$ at 734 nm because of chromatographic interferences. In this work the detector was set at 412 nm because it is the wavelength of maximum absorption of the ABTS cation. The presence of antioxidants, acting as radical scavengers, results in a reaction with $ABTS^{\bullet+}$

and a subsequent decrease in absorption detected as a negative peak at 412 nm.

Antioxidant compounds, even at low concentrations, could be quantified. The quantification limits with LC-UV detection varied from 2 pmol for chlorogenic acid to 541 pmol for epicatechin (Table 1). This high limit of quantification for epicatechin, compared with other compounds, was due to a high coefficient of variation at lower concentrations probably because it was monitored at 254 nm, which is not its optimal detection wavelength (280 nm). The quantification limits with LC-AOx detection varied from 54 pmol for epicatechin to 2575 pmol for ferulic acid (Table 1). The LC-UV detection was generally more sensitive than this of LC-AOx except for gallic acid and epicatechin.

Panels b and c of Figure 1 show chromatograms obtained with a standard mixture of phenolic compounds (30 $\mu$g/mL each) and their corresponding antioxidant activity. The upper part was obtained with direct UV detection at 254 nm, whereas the lower part was obtained with visible detection at 412 nm after post-column reaction. The combination of a delay coil of 7 m length, 0.5 mm internal diameter, with a flow rate of 0.5 mL/min gave a reaction time of about 1 min. This delay along with the passage through the coil explains the shift in retention time and the wider peaks obtained in the bottom chromatogram; however, it was necessary, in these conditions, to achieve a complete reaction with all antioxidants.[15] Using this method, standard phenolic compounds tested did not show equal antioxidant activities; in particular, *p*-hydroxybenzoic, vanillic, *p*-coumaric, *m*-coumaric, and *o*-coumaric acids showed no antioxidant activity at all or had an antioxidant activity too small to be detected with the LC-AOx. Among the active compounds, the relative antioxidant activity of these compounds was as follows: gallic acid > epicatechin > caffeic acid > catechin > sinapic acid > chlorogenic acid > protocatechuic acid > ferulic acid. This is in agreement with results obtained by Kim et al., who, using the same radical, showed the following antioxidant activity order: gallic acid > epicatechin > catechin > chlorogenic acid.[16] Gallic acid, with the best antioxidant response, was approximately 75 times more potent than ferulic acid, the least active compound.

**Extraction.** The extraction method accuracy was validated by monitoring the amount of phenolic compounds extracted over three consecutive extractions of wort. Concentrations of extracted phenolic compounds were given as mean ± standard deviation (SD) from at least three determinations ($n \geq 3$). Except with protocatechuic acid, chlorogenic acid, and epicatechin, for which recovery rates did not exceed 64 ± 9, 51 ± 6, and 49 ± 10%, respectively, three successive extractions were enough to obtain recovery rates above 75% (Table 2). Even if the liquid−liquid extraction with ethyl acetate did not allow a good extraction of each individual compound, 75% seems to be quite a good result for a multicompound extraction process. Today, this method remains the most widely used method for the extraction of polyphenols,[5,17−19] even if the extraction procedure is classical. Although we tested other methods, such as solid-phase extraction on wort, the results were less satisfactory when the whole range of compounds was considered (data not shown).[20] In view of the various compounds in the different extracts of beer, there were a lot of unknown compounds without data of recovery rate. Therefore, the recovery rate correction was not applied to the extraction method data. The recovery rate measurement was used to highlight the extraction method efficiency.

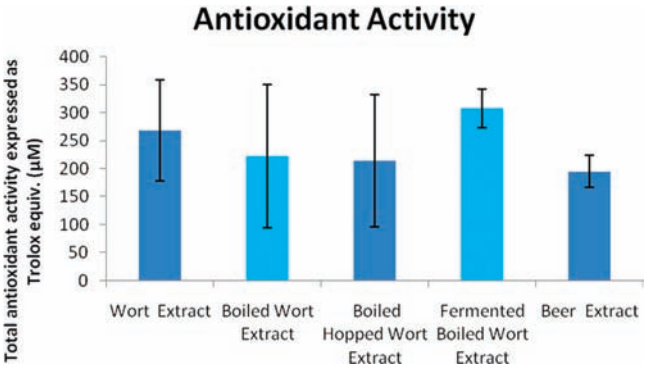


**Figure 3.** Total antioxidant activity at different steps of beer processing: wort extract, boiled hopped wort extract, and beer extract correspond to normal process; boiled wort extract and fermented boiled wort extract correspond to process excluding the hopping steps.

**Analysis of Beer Processing Compounds.** The LC-AOx method was applied to the separation and the evaluation of the antioxidant activity of compounds from extracts at different steps of beer processing: brewing, boiling, and fermentation. This allowed the direct monitoring of the antioxidant activity of polyphenolic fractions separated from extracts of wort, boiled wort, boiled hopped wort, fermented boiled wort, and beer. Wort, boiled hopped wort, and beer represented the normal processing of beer, whereas boiled wort and fermented boiled wort represented a process that would exclude the hopping step.

Figure 2 shows chromatograms obtained with ethyl acetate extracts of wort (a), boiled wort (b), boiled hopped wort (c), fermented boiled wort (d), and beer (e). The UV absorption chromatograms between wort (Figure 2a), boiled wort (Figure 2b), and fermented boiled wort (Figure 2d) were quite similar. With boiled hopped wort (Figure 2c) four peaks seemed in higher concentration than in the other profiles: b (12.5 min), c (13.8 min), d (14.2 min), and e (15.5 min). With beer (Figure 2e), the same peaks were present, but peak c (13.8 min) was 3 times higher than the boiled hopped wort one. This increase may be linked to a better extraction of compounds due to the presence of ethanol.

For each step, peak areas of chromatograms for the LC-AOx detection system were summed and represented as average ± SD from triplicates of three determinations (Figure 3). The total antioxidant activity results were expressed as Trolox equivalent ($\mu$M). It was affected neither by the brewing process nor by the fermentation process, because there was no significant difference ($p > 0.05$) between the different steps (Figure 3). The antioxidant activity profiles were quite similar for all chromatograms. With boiled hopped wort (Figure 2c) and beer (Figure 2e), the first antioxidant activity peak at 11 min (first arrow) was in a little bit higher concentration and a peak emerged at 14.9 min (second arrow). This peak corresponds to the one at 13.8 min in LC-UV profiles. These differences between chromatograms may be due to the increased content of natural compounds due to the depolymerization of antioxidant compounds during the process. It might not be due to the appearance of new polyphenols due to hop addition or fermentation. Indeed, hop extract was produced by $CO_2$ supercritical extraction. Because $CO_2$ is nonpolar, it is not a good solvent for polar polyphenols.[21] Moreover, hop extract did not show polyphenol content with LC-AOx method (data not shown).

Among the polyphenols with antioxidant activity, seven compounds could be identified using chromatographic standards: protocatechuic acid (2), catechin (4), chlorogenic acid (5),

**Table 3. Content and Antioxidant Activity of Antioxidant Compounds in Extracts at Different Stages of Beer Processing ($n$ = 9)**

| | wort extract | | boiled wort extract | | boiled hopped wort extract | | fermented boiled wort extract | | beer extract | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LC-UV concn ($\mu$M) | LC-AOx Trolox equiv ($\mu$M) | LC-UV concn ($\mu$M) | LC-AOx Trolox equiv ($\mu$M) | LC-UV concn ($\mu$M) | LC-AOx Trolox equiv ($\mu$M) | LC-UV concn ($\mu$M) | LC-AOx Trolox equiv ($\mu$M) | LC-UV concn ($\mu$M) | LC-AOx Trolox equiv ($\mu$M) |
| a, unknown | nq[a] | 24 ± 10 | nq | 32 ± 8 | nq | 53 ± 10 | nq | 45 ± 12 | nq | 60 ± 12 |
| b, unknown | nq | 8 ± 4 | nq | 1 ± 0 | nq | 4 ± 0 | nq | 8 ± 4 | nq | 8 ± 1 |
| c, unknown | nq | 5 ± 0 | nq | 7 ± 5 | nq | 12 ± 3 | nq | 8 ± 1 | nq | 5 ± 0 |
| d, unknown | nq | 49 ± 34 | nq | 73 ± 46 | nq | 35 ± 18 | nq | 58 ± 30 | nq | 15 ± 7 |
| e, unknown | nq | 8 ± 5 | nq | 22 ± 3 | nq | 10 ± 4 | nq | 10 ± 4 | nq | 20 ± 13 |
| protocatechuic acid | 21 ± 10 | 6 ± 2 | 7 ± 3 | 3 ± 1 | 17 ± 7 | 11 ± 5 | 9 ± 4 | 11 ± 3 | 27 ± 13 | 10 ± 5 |
| f, unknown | nq | 8 ± 3 | nq | 8 ± 8 | nq | 9 ± 0 | nq | 6 ± 1 | nq | 11 ± 8 |
| g, unknown | nq | 74 ± 51 | nq | 113 ± 76 | nq | 58 ± 40 | nq | 74 ± 38 | nq | 22 ± 17 |
| h, unknown | nq | 5 ± 3 | nd[b] | nd | nq | 7 ± 0 | nq | 4 ± 0 | nd | nd |
| *p*-hydroxybenzoic acid | 8 ± 2 | –[c] | 11 ± 3 | – | 7 ± 3 | – | 38 ± 15 | – | 18 ± 2 | – |
| i, unknown | nq | 5 ± 3 | nd | nd | nd | nd | nd | nd | nd | nd |
| catechin | 35 ± 18 | 38 ± 19 | 37 ± 18 | 47 ± 23 | 33 ± 10 | 44 ± 31 | 29 ± 12 | 43 ± 13 | 38 ± 21 | 32 ± 19 |
| chlorogenic acid | 4 ± 2 | 5 ± 3 | 5 ± 1 | nd | 6 ± 3 | 9 ± 1 | 13 ± 12 | 10 ± 0 | 11 ± 2 | nd |
| vanillic acid | 16 ± 10 | – | 19 ± 3 | – | 17 ± 7 | – | 34 ± 13 | – | 33 ± 5 | – |
| caffeic acid | 13 ± 5 | 15 ± 1 | 13 ± 3 | 17 ± 6 | 12 ± 4 | 15 ± 2 | 13 ± 5 | 14 ± 8 | 10 ± 6 | 11 ± 7 |
| j, unknown | nq | 8 | nq | 13 ± 2 | nq | 10 ± 2 | nq | 14 ± 6 | nq | 11 ± 5 |
| epicatechin | 35 ± 19 | 2 ± 1 | nd | nd | 14 ± 9 | 13 ± 1 | 49 ± 0 | 11 ± 0 | 19 ± 7 | 23 ± 3 |
| k, unknown | nd | nd | nd | nd | nq | 13 ± 7 | nq | 17 ± 2 | nd | nd |
| l, unknown | nd | nd | nd | nd | nq | 16 ± 0 | nd | nd | nd | nd |
| *p*-coumaric acid | 70 ± 31 | – | 84 ± 26 | – | 322 ± 86 | – | 45 ± 23 | – | 60 ± 12 | – |
| m, unknown | nq | 3 ± 1 | nq | 9 ± 0 | nq | 9 ± 2 | nq | 5 ± 1 | nq | 17 ± 9 |
| ferulic acid | 129 ± 52 | 8 ± 5 | 133 ± 23 | 11 ± 5 | 118 ± 49 | 9 ± 2 | 115 ± 49 | 12 ± 1 | 114 ± 11 | 11 ± 6 |
| sinapic acid | 18 ± 7 | 15 ± 8 | 22 ± 3 | 21 ± 6 | 28 ± 10 | 20 ± 4 | 10 ± 4 | 21 ± 7 | 11 ± 2 | 16 ± 9 |
| *m*-coumaric acid | 7 ± 5 | – | 12 ± 5 | – | 6 ± 2 | – | 11 ± 5 | – | 14 ± 9 | – |
| n, unknown | nq | 5 ± 1 | nq | 10 ± 6 | nq | 7 ± 3 | nq | 10 ± 4 | nq | 10 ± 8 |
| o, unknown | nq | 7 ± 2 | nq | 10 ± 6 | nq | 8 ± 4 | nq | 9 ± 4 | nq | 12 ± 4 |

[a] nq, not quantified (no standards). [b] nd, not detected. [c] –, no antioxidant activity.

caffeic acid (7), epicatechin (8), ferulic acid (10), and sinapic acid (11). The largest antioxidant contribution came from catechin, caffeic acid, ferulic acid, sinapic acid, and three other compounds (a, d, and g), which could not be identified using chromatographic standards (Table 3). The total antioxidant activity of each extract was measured, and these three compounds, present in all extracts, accounted for 64 ± 4% of the total. Catechin was important to beer antioxidant activity because it made considerable contributions to the antioxidant activity of beer. Compounds with flavonoid structure such as catechin generally showed higher antioxidant activity than nonflavonoid compounds.[14] Whittle et al. reported the general observation that the higher the content of gallocatechin in gallocatechin polymers is, the earlier the compounds elute.[22] Due to the high antioxidant activity of compounds a, d, and g, and on the basis of their retention times, they are probably compounds with flavanoid structure such as polymers of gallocatechin.

The LC-AOx method was used for the determination of phenolic compounds during beer processing. For reactive antioxidants, this method may prove to be more sensitive than the classical HPLC with UV detection. It is also more specific because it is intended not only to separate and detect polyphenols, but also to determine their potential functional interest, namely, their antioxidant activity. Unknown antioxidant compounds could be detected, which accounted for the larger part of the antioxidant activity of the extracts. Work is underway to identify these unknown compounds.

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