Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1054 (2004) 175–180
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Development of a rapid method based on solid-phase extraction and liquid chromatography with ultraviolet absorbance detection for the determination of polyphenols in alcohol-free beers

A. Alonso García, B. Cancho Grande, J. Simal Gándara*

Nutrition and Bromatology Group, Analytical and Food Chemistry Department, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, 32004 Ourense, Spain

Available online 11 September 2004

Abstract

An analytical method based on solid-phase extraction (SPE) and followed by liquid chromatographic separation and ultraviolet detection (HPLC–UV) is proposed for the determination of 10 phenolic compounds which participate on beer stability and sensory properties in alcohol-free beers. Acetonitrile was found to be the most appropriate solvent for the elution of polyphenolic compounds adsorbed on C_{18} cartridges. The performance of the method was assessed by the evaluation of parameters such as absolute recovery (generally higher than 60%), repeatability (lower than 10%), linearity (r^2 higher than 0.993) and limits of quantitation (ranging from 1 to 37 μg/L); no matrix effects were observed. The polyphenol content of different Spanish alcohol-free beers is presented. Five phenolic compounds such as protocatechuic, *p*-coumaric, ferulic, caffeic acids, and (+)-catechin were identified at levels lower than 10 mg/L.

Keywords: Polyphenols; Beers

1. Introduction

Alcohol-free beers are recommended for specific groups of people such as the pregnant women, sporting professionals, people with cardiovascular and hepatic pathologies, medicated people, etc. These beverages have a complex mixture of phenolic compounds extracted from malt and hops which have been shown to have useful antioxidant properties [1]. Moreover, three groups of polyphenols are responsible for beer flavor and physical stability [1–3] Simple polyphenols derived from hydroxybenzoic acids (gallic acid, protocatechuic acid, syringic acid, etc.) and hydroxycinnamic acids (ferulic acid, *p*-coumaric acid, caffeic acid, syringic acid, etc.) are extracted mostly from malt but also present in small amounts in hops. Flavonols (quercetin, kaempferol, etc.) derived mostly from hops. Flavan-3-ols, including monomers such as (+)-catechin and (−)-epicatechin, dimers (prodelphinidin B3 and procyanidin B3), trimers (procyanidin C2), up to higher-molecular weight flavonoid-derived tannins, arise equally from malt and hops. The final content of phenolic components of beer depends on both the raw materials and the brewing process.

Phenolic flavors are originated from phenolic acids naturally found in malt. These acids have a great ability to undergo decarboxylation, either by thermal fragmentation or through the activity of microorganisms. Strains of yeast with the right enzymes transform ferulic acid and *p*-coumaric acid into vinyil derivates or into substituted phenyl propionic acids [4,5]. As a consequence, highly flavor-active phenols are produced which may be appreciated in certain beers and in others they may be regarded as distasteful. Flavanols are assumed to be most frequent cause of haze in beer due to protein–polyphenol interactions [6]. The hydrophilic characteristics of proteins and the hydrophobic characteristics of polyphenols combine to form surfactant-like molecules.

* Corresponding author. Tel.: +34 988387000; fax: +34 988387001.
E-mail address: jsimal@uvigo.es (J. Simal Gándara).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.07.092

In sufficient concentration, these molecules from a polydisperse suspension of micelles that cause a hazy look in the beer. Oxygen and temperature catalyze the reaction between the proteins and polyphenols. Clear beverages are typically stabilized to delay the onset of protein–polyphenol haze formation. Lowering the concentration of the phenolic proanthocyanidins in beer, e.g., by cold filtration or a treatment with polyvinylpolypyrrolidone (PVPP) can efficiently delay the formation of haze during storage [7,8].

Rapid analytical methods are necessary for the quality control department of beer producers to evaluate phenolic compounds that may adversely affect beer flavor and stability, what is of practical interest. Analytical methods for determining phenolic compounds in wort and beer are limited. Some authors determined phenolic compounds in beer matrices by direct injection in HPLC, after filtration, since fermentable sugars, dextrins and organic acids do not interfere with their chromatographic response [9–11]. De Pascual-Teresa et al. [12] proposed an HPLC separation and on-line detection by diode-array spectroscopy after a chemical reaction with *p*-dimethylaminocynnamaldehyde (DMACA). Extraction of phenolic compounds in beers is also performed by liquid–liquid extraction (LLE) with organic solvents like *n*-hexane, isooctane, ethyl acetate and acetone/water [13–16]. Organic acids in beer were recovered by Belke and Irwin [17] with an anion-exchange resin and then were converted to their methyl esters by treatment of the resin with BF_3-methanol. Meanwhile the solid-phase extraction (SPE) is the common technique used for preconcentration and purification prior to HPLC separation of phenolic compounds in wines [18–20], this extraction technique has been not applied in beers. Separation of phenolic compounds in beer was performed commonly by reverse-liquid chromatography followed by ultraviolet [14,21], photodiode array [22,23], fluorimetric [15], electrochemical [14,21,24,25] or mass spectometric detection [16].

A method based on solid-phase extraction and followed by liquid chromatographic separation with ultraviolet detection (HPLC–UV) is presented as an analytical tool useful in quality control in the brewing industry for the determination of phenolic acids such as cafeic acid, *p*-coumaric acid, gallic acid, gentisic acid, ferulic acid and salycilic acid, flavonols such as quercetin, and flavanols such as (+)-catechin and (−)-epicatechin. Chemical structures of target compounds are shown in Table 1. The method was applied to the analysis of these compounds in alcohol-free beers to quantitatively measure these components in beer.

2. Experimental

2.1. Chemicals, solutions and disposables

Standards of caffeic acid (97%) CAS No. [331-39-5], *p*-coumaric acid (98%) [501-98-4], ferulic acid (99%) [537-98-4], gallic acid (97%) [149-91-7], gentisic acid (98%) [490-79-9], salicylic acid (99%) [69-72-7], protocatechuic acid (97%) [99-50-3], (+)-catechin hydrate (98%) [225937-10-0], quercetin dihydrate (98%) [6151-25-3] were purchased from Aldrich (Milwaukee, USA). (−)-Epicatechin (90%) [490-46-0] was purchased from Fluka (Buchs, Switzerland). Solvents such as acetone, ethyl acetate, dichloromethane, hexane, methanol and water for liquid chromatography were purchased from Merck (Darmstadt, Germany); acetonitrile for instrumental analysis was instead from Panreac (Barcelona, Spain). Other reagents used was anhydrous sodium sulphate ACS-ISO for analysis and hydrochloric acid 37% from Panreac (Spain).

A stock standard solution (ca. 500 mg/L) of each phenolic compound was prepared in methanol by weighing approximately 0.025 g of the analyte into a 50 mL volumetric flask and diluting to volume. An intermediary mixed standard solution was prepared by dilution in methanol of the stock standard solutions to give a concentration of ca. 50 mg/L for each polyphenol. All standard solutions were stored in the dark at 4 °C and were stable for at least three months.

Waters 500 mg Sep-Pak C_{18} Plus cartridges (Mildford, USA) were used as solid-phase extraction minicolumns for purification and concentration. A visiprep solid-phase extraction vacuum manifold from Supelco (Bellefonte, PA, USA) is used to simultaneously process up to 24 SPE tubes. The visidry drying attachment (Supelco) is used to dry up to 24 SPE tubes at one time, and can be used with any inert gas supply. It is also useful for evaporating and concentrating recovered eluates. Nitrogen C-50 of analytical quality was supplied by Carburos Metálicos (Vigo, Spain). SPE organic eluates were placed into round-bottom flasks from Schott Duran (Hattenbergstrabe, Germany) prior to be evaporated in a Heidolph WB 2000 vacuum rotary evaporator (Cinnamiuson, Germany). Final extracts were placed into 350 μL inserts in 2 mL vials from Supelco and homogenization was achieved by vortex agitation with a Heidolph Reax Top Apparatus (Germany).

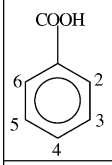
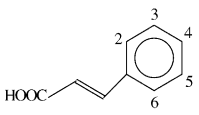
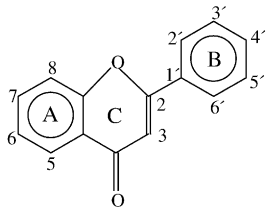
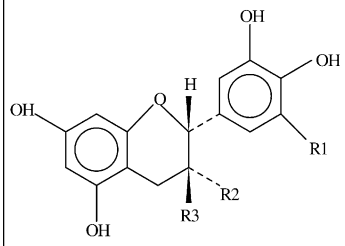
Nine non-alcoholic beers (labelled as A–I) produced for different manufacturers in Spain and used for characterizating the proposed method as well as for screening the presence of these phenolic compounds, were purchased at local markets in Ourense, Spain.

2.2. Chromatographic system and operating conditions

The analysis was performed on a Thermo HPLC system equipped with a SCM1000 vacuum membrane degasser, a P200 gradient pump, an AS1000 autosampler and a UVIS20 ultraviolet detector linked to a PC computer running the software program ChromCard version 1.21 (ThermoQuest, Italy).

The analytical column (150 × 4.6 mm i.d.) used was a Waters Symmetry 5 μm C_{18} (USA). The guard column (50 × 4.6 mm i.d.) was packed with dry 40 μm Pelliguard LC-18 (Supelco). For HPLC analysis, an aliquot (20 μL) was injected into the columns and eluted at room temperature

Table 1
Chemical structures of polyphenols

Benzoic acids 	Salicylic acid (2-OH) Gentisic acid (2,5-OH) Protocatechuic acid (3,4-OH) Gallic acid (3,4,5-OH)
Cinnamic acids 	*p*-coumaric acid(4-OH) Caffeic acid(3,4-OH) Ferulic acid (4-OH,3-OCH$_3$)
Flavones 	Quercetin (3,5,7,3´,4´-OH)
Catechins 	(+)-catechin (R$_1$=H, R$_2$=H, R$_3$=OH) (-)-epicatechin (R$_1$=H, R$_2$=OH, R$_3$=H)

with a constant flow rate of 1.0 mL/min at the following gradient conditions for the mobile phase – acid methanol (1% acetic acid) (A): acid water (1% acetic acid) (B): A:B (10:90) for 5 min, changed to A:B (50:50) for 30 min and held for 8 min; changed to A:B (10:90) for 1 min and held for 14 min. The mobile phase was acidified to guarantee total protonation of the studied compounds. For UV detection, a wavelength program was optimized to monitorize phenolic compounds at their respective maximum absorbance wavelength, as follows: λ: 280 nm and held for 12.7 min; changed to λ: 330 nm and held for 2 min; changed to λ: 306 nm and held for 2.9 min; changed to λ: 280 nm and held for 3.1 min; changed to λ: 306 nm and held for 15.3 min; and changed to λ: 253 nm and held for 5 min. Detection and quantification was done at 253 nm for quercetin; at 280 nm for gallic acid, (+)-catechin and (−)-epicatechin; at 306 nm for caffeic acid, *p*-coumaric acid and salycilic acid; and at 330 nm for gentisic acid.

2.3. Polyphenols extraction and purification

A 500 mg C_{18} Sep-Pak cartridge was conditioned with 5 mL of methanol followed by 10 mL of water without allowing the cartridge to dry out. An aliquot of the beer sample (25 mL), previously acidified to pH 1.5 with hydrochloric acid 37%, was passed through the cartridge. The cartridge was then gently dried by blowing nitrogen for 20 min. Subsequently, phenolic compounds were eluted by 12 mL of acetonitrile. The organic eluate was transferred to a 25 mL round-bottomed flask and evaporated to dryness on the rotary evaporator. The residue obtained was dissolved in 1 mL of methanol:water water (50:50, acidified at 1% with acetic acid). Homogenization of the final extract was achieved with vortex agitation prior to the chromatographic analysis.

3. Results and discussion

3.1. Method optimization

To remove beer matrix interferences, the purification efficiency of 360 and 500 mg C_{18} sorbents was tested. Commercial alcohol-free beer samples (labelled as A), previously degassed and acidified to pH 1.5, were spiked at 5 mg/L level with the target phenolic compounds. After equilibration for 5 min prior to extraction, spiked beer samples (10 mL) were processed according to the procedure described. Ethyl acetate (10 mL) was used for eluting the sorbent cartridges. Triplicate analyses were performed for each cartridge. The experimental results shown that 500 mg C_{18} was more effective in removing interfering compounds and more quantitative in recovering most of the studied phenolic compounds, as can be seen in Table 2. The organic

Table 2
Parameters optimized in the extraction and purification process expressed as absolute recovery (%) ± relative standard deviation (%)

	Gallic acid	Protocatechuic. acid	(+)-Catechin	Gentisic acid	Caffeic acid	(−)-Epicatechin	*p*-Coumaric acid	Ferulic acid	Salicylic acid	Quercetin
C_{18} type										
360 mg	2 ± 6	9 ± 13	14 ± 2	16 ± 3	28 ± 2	25 ± 10	69 ± 4	72 ± 2	54 ± <1	91 ± 2
500 mg	8 ± 10	18 ± 4	25 ± 4	31 ± 9	59 ± <1	52 ± 2	106 ± 4	94 ± <1	94 ± 2	79 ± <1
Elution solvent										
Ethyl acetate	8 ± 10	18 ± 4	25 ± 4	31 ± 9	59 ± <1	52 ± 2	106 ± 4	94 ± <1	94 ± 2	79 ± <1
Acetone	18 ± 15	8 ± 10	36 ± 10	29 ± 7	63 ± 8	66 ± 7	115 ± 2	92 ± 2	100 ± 4	91 ± 3
Acetonitrile	6 ± 12	33 ± 5	61 ± 9	50 ± 9	63 ± 4	66 ± 7	88 ± 3	100 ± 2	100 ± 6	92 ± 6
CO_2 removal										
No	6 ± 12	33 ± 5	61 ± 9	50 ± 9	63 ± 4	66 ± 7	88 ± 3	100 ± 2	100 ± 6	92 ± 6
Yes	6 ± 10	34 ± 8	50 ± 6	31 ± 18	62 ± 4	59 ± 2	86 ± 3	93 ± 3	83 ± 7	85 ± 4

(n = 2) determinations.

solvent for cartridge elution was then optimized by analysis of alcohol-free beer samples (10 mL), spiked at 5 mg/L level. SPE with the selective sorbent cartridge and elution (10 mL) with hexane, dichloromethane, ethyl acetate, acetonitrile, methanol and acetone was tested. Triplicate analyses were performed for each solvent. The elution efficiency of hexane, dichloromethane and methanol was lower than the obtained with ethyl acetate, acetonitrile and acetone. Acetonitrile allowed to obtain chromatograms cleaner than acetone and ethyl acetate and a volume of 12 mL of acetonitrile allowed to guarantee elution of the retained phenolic compounds (see Table 2). To simplify the extraction procedure, it was tested whether or not the carbon dioxide elimination step could be removed (see Table 2). No significant differences were found when the gas was present or previously removed. To increase the sensitivity of the method, higher volumes of beer (10–25 mL) can be used.

3.2. Method performance

Different commercial alcohol-free beer samples (A–C) purchased at different local markets were examined to study the matrix effect. All these beer samples were spiked by triplicate at a level of 2 mg/L, extracted and analyzed following the experimental procedure described. Beer sample A was also spiked at different levels ranging from 0.1 to 8 mg/L (n = 5) to construct method calibration lines by plotting polyphenol areas versus the added (Table 3). No significant differences on polyphenols recovery at the 95% probability level (α = 0.05) were found for the different beers spiked at the same polyphenol level. As a consequence, the quantitation process is not affected by the differences in beer matrices and method calibration can be performed by spiking different beer samples.

Quality parameters such as recovery values, repeatability, linearity and limits of detection (LOD) and quantitation (LOQ) were evaluated (Table 3**)**. For this purpose, commercial alcohol-free beer A samples were previously fortified with phenolic compounds (2 mg/L) and treated following the experimental conditions described. Analysis of the unspiked beer gave response at the retention time of some of the studied polyphenols; their contributions in the blank were substracted to estimate spiking recoveries. The recovery and repeatability of the method was assessed by analyzing seven spiked alcohol-free beer samples in the same day (ca. 2 mg/L of each phenolic compound). The relative standard deviation (R.S.D.%) was lower than 10%. These values show the good

Table 3
Repeatability, absolute recovery (%), method linear dynamic range, determination coefficient (r^2), limit of detection (LOD) and quantitation (LOQ) of the optimized method for the determination of phenolic compounds levels in alcohol-free beers

Phenolic compounds	Repeatability[a] ± R.S.D. (%)	Absolute[a] recovery (%)	Method Linear range[b] (mg/L)	Determination coefficient (r^2)	LOD[c] (mg/L)	LOQ[c] (mg/L)
Gallic acid	10	6[d]	0.3–8	0.993	0.20	0.34
Protocatechuic acid	5	33	0.1–4	0.998	0.02	0.04
(+)-Catechin	8	61	0.1–4	0.993	0.04	0.07
Gentisic acid	9	50	0.4–4	0.993	0.14	0.37
Caffeic acid	4	63	0.2–4	0.993	0.08	0.19
(−)-Epicatechin	7	66	0.1–4	0.993	0.08	0.11
p-Coumaric acid	3	88	0.1–2	0.993	0.04	0.07
Ferulic acid	2	100	0.1–4	0.998	<0.01	0.01
Salicylic acid	6	100	0.1–8	0.999	0.07	0.10
Quercetin	6	92	0.2–8	0.998	0.11	0.24

[a] n = 7.
[b] n = 6 determinations.
[c] n = 5.
[d] Low recovery due to its polarity.

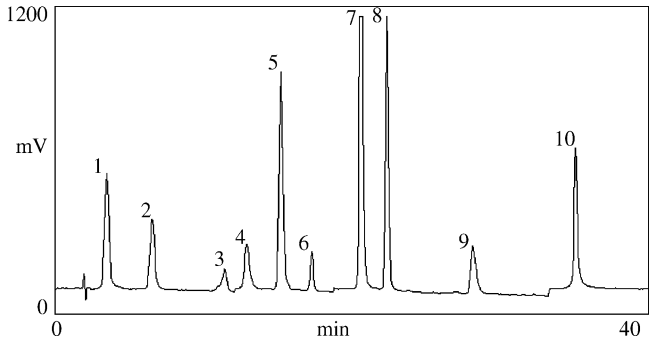


Fig. 1. HPLC–UV chromatogram for a polyphenolic mix standard solution (5 mg/L, in methanol. Peaks: (1) gallic acid, (2) protocatechuic acid, (3) (+)-catechin, (4) gentisic acid, (5) caffeic acid, (6) (−)-epicatechin, (7) *p*-coumaric acid, (8) ferulic acid, (9) salicylic acid, (10) quercetin.

precision of the method proposed. On the other hand, absolute recoveries, those measured against polyphenol standard solutions directly injected into the chromatographic column, are higher than 60% for practically all target compounds except for gallic, protocatechuic and gentisic acids. The lowest recovery was found for gallic acid, which is the most polar polyphenol, and by this reason could be not retained in the nonpolar C_{18} cartridges used for extraction. To correct for the absolute recovery below hundred percent, method calibration was done with the line obtained submitting fortified beers to the complete sample treatment and analysis. Consequently, relative recovery was about hundred percent because all standard-spiked beer samples (those for calibration purposes and those for polyphenols measurement) were processed in the same way and the absolute recovery can be considered constant for all polyphenols.

Limits of detection and quantitation were evaluated on the basis of the signal obtained with the analysis of unfortified alcohol-free beers samples ($n = 7$), following the recommendations of the American Chemical Society [26]. LOD and LOQ were defined as the concentration of the analyte that produced a signal-to-noise ratio of three and ten, respectively; they were lower than 0.2 and 0.4 mg/L, respectively, for all target compounds. A chromatogram of a polyphenol standard solution is shown in Fig. 1.

3.3. Polyphenol content in commercial alcoholic-free beers

As application of the proposed method, nine alcoholic-free beer samples produced in different Spanish breweries were analyzed. The identification of the peaks was carried out by their retention times in comparison with standards and by the method of standard addition to the samples, but also comparing the UV spectra in samples and standards by using a photodiode array detector. The values of the studied polyphenols in alcohol-free beers are shown in Table 4. Total content ranged from 3.5 to 8.5 mg/L, except for beer E with a lower phenolic content (0.8 mg/L). Ferulic (ranging from 0.7 to 2.3 mg/L), *p*-coumaric (0.1–0.7 mg/L) and caffeic acid (0.2–0.4 mg/L) were the hydroxycinnamic acids identified; protocatechuic acid (0.7–5.1 mg/L) was the only hydroxybenzoic acid identified; and finally, catechin monomer (+)-catechin (0.3–4.5 mg/L) was also present. With regards to individual compounds, protocatechuic acid was the most abundant phenolic compound found, followed by (+)-catechin, ferulic acid and *p*-coumaric. Ferulic acid concentrations are almost higher than *p*-coumaric acid concentrations since ferulic acid is formed from *p*-coumaric acid via the shikimic acid pathway [27]. Caffeic acid and epicatechin were identified in some of the beer samples. Quercetin, gallic acid, gentisic and salicylic acids were not identified in the commercial beers.

The levels found agree with phenolic concentrations determined by other authors in literature. McMurrough et al. [13] determined the total content of phenolic acids in beers brewed in Ireland and they ranged between 5 and 8 mg/L being vanillic, *p*-coumaric and ferulic acids the predominant phenolic acids. Hayes et al. [14] determined the phenolic compounds commonly found in Irish-brewed beers: as benzoic acid derivatives, protocatechuic and gallic acids were identified but not quantified; as cinnamic acid derivatives, caffeic (ranging from 0.13 to 0.30 mg/L), *p*-coumaric (0.57–0.92 mg/L) and ferulic acid (1.05–1.90 mg/L) were found; and finally, (+)-catechin (N.D.-0.82 mg/L) and (−)-epicatechin (0.10–0.25 mg/L) were also quantified. De Pascual-Teresa et al. [12] determined the individual content of 15 flavonols in 56 different Spanish foodstuffs and beverages (tea, wine

Table 4
Polyphenol content (average ± standard deviation; mg/L) in commercial alcohol-free samples

	A	B	C	D	E	F	G	H	I
Gallic acid	–	–	–	–	–	–	–	–	–
Protocatechuic acid	0.66 ± 0.04	2.7 ± 0.2	3.4 ± 0.2	2.7 ± 0.2	–	4.7 ± 0.3	0.93 ± 0.05	5.1 ± 0.3	4.5 ± 0.3
(+)-Catechin	0.31 ± 0.03	1.0 ± 0.1	4.5 ± 0.4	–	–	–	2.8 ± 0.2	0.42 ± 0.03	0.77 ± 0.06
Gentisic acid	–	–	–	–	–	–	–	–	–
Caffeic acid	–	–	0.24 ± 0.01	0.32 ± 0.02	–	0.41 ± 0.02	0.19 ± 0.01	–	–
(−)-Epicatechin	–	–	–	–	–	0.18 ± 0.01	0.21 ± 0.01	–	0.22 ± 0.02
p-Coumaric acid	0.22 ± 0.01	0.49 ± 0.02	0.41 ± 0.02	0.23 ± 0.01	0.11 ± 0.01	0.73 ± 0.03	0.64 ± 0.02	0.62 ± 0.02	0.38 ± 0.01
Ferulic acid	2.3 ± 0.1	1.3 ± 0.1	–	1.5 ± 0.1	0.68 ± 0.02	2.3 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	0.66 ±0.02
Salicylic acid	–	–	–	–	–	0.13 ± 0.01	–	–	–
Quercetin	–	–	–	<LOQ	–	–	–	–	–
Total	3.5	5.5	8.6	4.8	0.8	8.5	6.1	7.6	6.5

$n = 2$ determinations; – <LOD.

and beer); (+)-catechin (7.3 mg/L) and (−)-epicatechin were identified in beer samples. Floridi et al. [11] determined 19 phenolic compounds in beer; values, average values of 23 different samples were 0.6 mg/L for gallic acid, 0.84 mg/L for protocatechuic acid, 0.4 mg/L for gentisic acid, 0.6 mg/L for caffeic acid, 1.4 mg/L for *p*-coumaric acid, 2.4 mg/L for ferulic acid and 2.9 mg/L for salicylic acid. Bartolome et al. [28] compared the phenolic compounds of several commercial alcohol-free and standard beers; the levels of polyphenol components in alcohol-free beers were lower than the values for standard beers and this is attributable to differences in the duration of fermentation and the yeast strains employed in brewing alcohol-free beers and to losses brought by the dealcoholization processes employed affecting to polyphenols such as *p*-coumaric, caffeic, vanillic acids, etc. No distasteful flavors were detected in the commercial beers considered. McMurrough et al. [13] affirmed that the flavor threshold for a nine components phenolic acid mixture was 50–100 mg/L. No haze in beer was observed probably due to methods for partial removal of polyphenols in the mashing process by addition of hexamethylenetetramine or sulfite [10], by cold filtration or treatment with PVPP [7,8].

4. Conclusions

The optimized method is presented as a research analytical tool for the routine control of the composition of alcohol-free beers. It was also successfully tested with standard beers after removing the ethanol by rotary evaporation. The use of 500 mg C_{18} SPE cartridges and acetonitrile as elution solvent allows their determination without interferences. The method has good linearity, precision and sensitivity. In general, except for gallic and protocatechuic acid recovery is good; it is possible to correct for the recoveries lower than 60% since these can be considered constant. Its application to commercial alcohol-free beers allows to confirm that the levels of polyphenols measured does not affect flavor and stability of beer.

References

[1] P. Goupy, M. Hughes, P. Boivin, M.J. Amiot, J. Sci. Food Agric. 79 (12) (1999) 1625.
[2] F. Shahidi, M. Naczk (Eds.), Food Phenolic: Sources, Chemistry, Effects, Applications, Technomic Publishing Co. Inc., Lancaster, PA, 1995, pp. 10–13.
[3] A. de Stefano, L. Montanari, Alcologia 8 (1996) 43.
[4] I. McMurrough, D. Madigan, D. Donnelly, J. Hurley, A.M. Doyly, G. Hennigan, N. McNulty, J. Inst. Brew. 102 (1996) 327.
[5] L. Barthelmebs, C. Divies, J.F. Cavin, Appl. Environ. Microbiol. 66 (8) (2000) 3368.
[6] J.S. Hough, D.E. Briggs, R. Stevens, T.M. Young, , in: Malting and Brewing Science, vol. 2, second ed., Chapman and Hall, London, 1982.
[7] I. McMurrough, D. Madigan, R.J. Kelly, M.R. Smyth, J. Am. Soc. Brew. Chem. 54 (1996) 141.
[8] I. McMurrough, D. Madigan, R.J. Kelly, J. Am. Soc. Brew. Chem. 55 (1997) 38.
[9] D. Madigan, I. McMurrough, M.R. Smyth, J. Am. Soc. Brew. Chem. 52 (4) (1994) 152.
[10] M.L. Andersen, L.H. Skibsted, J. Agric. Food Chem. 49 (2001) 5232.
[11] S. Floridi, L. Montanari, O. Marconi, P. Fantozzi, J. Agric. Food Chem. 51 (2003) 1548.
[12] S. De Pascual-Teresa, C. Santos-Buelga, J.C. Rivas-Gonzalo, J. Agric. Food Chem. 48 (2000) 5331.
[13] I. McMurrough, G.P. Roche, K.G. Cleary, J. Inst. Brew. 90 (3) (1984) 181.
[14] P.J. Hayes, M.R. Smyth, I. McMurrough, Analyst 112 (9) (1987) 1205.
[15] F. García-Sánchez, C. Carnero, A. Heredia, J. Agric. Food Chem. 36 (1) (1988) 80.
[16] N. Whittle, H. Eldridge, J. Bartley, O. Gregory, J. Inst. Brew. 105 (2) (1999) 89.
[17] C.J. Belke, A.J. Irwin, J. Am. Soc. Brew. Chem. 50 (1) (1992) 26.
[18] C. Betés-Saura, C. Andrés-Lacueva, R.M. Lamuela-Raventós, J. Agric. Food Chem. 44 (1996) 3040.
[19] D.A. Guillén, F. Merello, C.G. Barroso, J.A. Pérez-Bustamante, J. Agric. Food Chem. 45 (1997) 403.
[20] S. Karagiannis, A. Economou, P. Lanaridis, J. Agric. Food Chem. 48 (2000) 5369.
[21] P.J. Hayes, M.R. Smyth, I. McMurrough, Analyst 112 (9) (1987) 1197.
[22] N. Es-Safi, H. Fulcrand, V. Cheynier, M. Moutounet, J. Agric. Food Chem. 47 (1999) 2088.
[23] L. Montanari, G. Perretti, F. Patella, A. Giudi, P. Fantozzi, Lebensm. Wiss. Technol. 32 (1999) 1.
[24] C. Lunte, J. Wheeler, W. Heineman, Analyst 113 (1) (1988) 95.
[25] P. Jandera, L. Grynova, G. Skopova, CHEMagazin 12 (6) (2002) 8.
[26] American Chemical Society (ACS) Subbcommite on Enviromental Analytical Chemistry, Anal. Chem. 52 (1980) 2242.
[27] T.M. Kenyhercz, P.T. Kissinger, J. Agric. Food Chem. 25 (1977) 959.
[28] B. Bartolomé, A. Pena-Neira, C. Gómez-Cordoves, Eur. Food Res. Technol. 210 (6) (2000) 419.