Solid-Phase Microextraction for the Analysis of Some Alcohols and Esters in Beer: Comparison with Static Headspace Method

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Headspace solid-phase microextraction (SPME) has been used for the determination of 12 alcohols and esters in beer. SPME analysis parameters using polyacrylate fiber have been defined and compared with the static headspace (SHS) method used as a reference. Limits of detection, linearity, and repeatability of both methods have been determined using standard solutions. Limits of detection of SPME were generally lower than of the SHS method, and addition of salt enhanced SPME adsorption. Both methods were characterized by high repeatability and good linearity. Results of beer analyses obtained by using these two methods were highly correlated, which indicates the possibility of SPME application as an inexpensive alternative to automated SHS in the analysis of higher alcohols and esters in beer.

Keywords: *SPME; static headspace; beer alcohols; esters*

INTRODUCTION

Beer flavor, being a combination of odor and taste impressions, is a crucial factor in consumer acceptance of this product. A list of beer constituents comprises >800 compounds, many of them contributing to its flavor characteristic. Meilgaard (1982), in his thorough paper, discussed the influence of chemical composition on the flavor of beer. Depending on beer type the following "flavor groups" play a diversified role in flavor perception: bitterness, alcoholic flavor, carbonation, hop character, caramel flavor, fruity/estery flavor, sweetness, acidity, and dimethyl sulfide flavor (Meilgaard, 1982).

Higher alcohols and esters fall into a group of volatile constituents, which form the major part of beer flavor. Low concentrations of these compounds contribute positively to beer "bouquet," whereas their concentrations exceeding odor thresholds may be a cause of distinctive off-odors and contribute to the unacceptable taste of beer. Esters are often characterized as possessing "banana flavor" (isoamyl acetate) or "apple flavor" (ethyl hexanoate, ethyl octanoate). Ethyl acetate is often characterized by an "adhesive" flavor note. Propanol, isobutanol, and isoamyl alcohol are assumed to have estery, fusel odors (Meilgaard, 1982; Stempfl, 1995; Diedericks, 1996). In discussions of flavor impression, the interactions between odor compounds, mainly synergism and masking effects, should also be taken into consideration.

Higher alcohols and esters are byproducts of ethanol fermentation, and their yield during beer production is dependent upon several factors. Higher alcohols are formed in the Ehrlich pathway, where wort amino acids are taken up by the yeast and, after transamination to α-keto acids and their subsequent decarboxylation, transferred to the corresponding alcohols. For example, isoamyl alcohol and isobutanol originate from, respectively, leucine and valine biosynthetic pathways (Nykänen and Suomalainen, 1983). Most esters found in beer are products of yeast metabolism. For ester synthesis two substrates, alcohol and acyl-coenzyme A, are used and the reaction is catalyzed by an ester synthase or acyl transferase (Berry, 1988; Nykänen and Suomalainen, 1983). During the brewing process higher alcohol and ester production is influenced mainly by the wort composition, fermentation parameters, and yeast strains.

For the determination of higher alcohols and esters in beer, well-established methods based on a static headspace analysis (SHS) are used worldwide, being both reliable and predisposed to automatization (Drawert, 1982; Diedericks, 1996).

Solid-phase microextraction (SPME) is a relatively novel, solventless method of volatile extraction from gaseous, solid, or liquid phase (Arthur and Pawliszyn, 1990; Zhang and Pawliszyn, 1993; Ibañez and Bernhard, 1996; Pawliszyn, 1997). Developed by Pawliszyn's group in the early 1990s, it has proven to be a fast and reliable method in environmental analyses and it is gaining recognition in food analysis as well. This technique has been utilized in such areas of food/flavor chemistry as pesticide determination in wine (Urruty et al., 1997), monitoring of volatiles of apple fruit (Song et al., 1997), detection of flavor additives in tobacco products (Clark and Bunch, 1997) and volatile metabolites emitted by microorganisms (Nilsson et al., 1996), and characterization of vodkas (Ng et al., 1996).

The objective of this study was to assess the usefulness of an SPME method in the quantitative analysis of higher alcohols and esters in beer and to compare this low-cost technique to one of the automated SHS methods of determination of these compounds (Diedericks, 1996).

EXPERIMENTAL PROCEDURES

Samples and Chemicals. Standards of analyzed compounds (ethyl acetate, isobutyl acetate, ethyl butyrate, butyl acetate, isoamyl acetate, ethyl caproate, ethyl caprylate,

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propanol, isobutanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and 1-pentanol) were purchased from Sigma-Aldrich and were of >98% purity. Bottles of beer, Pilsner type, were purchased at a local store.

Standard and Sample Preparation. For method performance evaluation a standard solution of 12 alcohols and esters was used. A standard stock solution was prepared by dissolving analyzed compounds in 96% ethanol and was stored in 4 °C. To prepare the standard mixture solution for method evaluation, 9.5 mL of deionized water was added to a 20 mL headspace vial, followed by the addition of 30 μL of 1-pentanol (5 mg/mL) as an internal standard and 25 μL of stock standard solution that resulted in the following concentrations: ethyl acetate, 7.37 mg/L; isobutyl acetate, 1.26 mg/L; propanol, 7.00 mg/L; ethyl butyrate, 1.08 mg/L; butyl acetate, 1.10 mg/L; isobutanol, 7.49 mg/L; isoamyl acetate, 7.39 mg/L; isoamyl alcohols, 20.99 mg/L; ethyl caproate, 1.37 mg/L; and ethyl caprylate, 2.02 mg/L. The volume was then filled up to 10 mL and the vial capped with a Teflon-lined cap.

To prepare the beer samples after a bottle had been opened, 100 mL of beer was poured into a 300 mL Erlenmeyer flask and delicately shaken for 10 s; 9.970 mL was then transferred to a headspace vial. After addition of 30 μL of internal standard, the vial was capped. Samples prepared in a way described above were used for both SHS and SPME analyses.

SHS Analysis. Analyses were done on a Hewlett-Packard HP 6890 gas chromatograph with a split/splitless injector and an FID detector. For the SHS analyses Hewlett-Packard headspace sampler HP 7694 was used. Compounds of interest were resolved on a Stabilwax (Restec, USA) capillary column (30 m × 320 μm i.d. × 1 μm) in the following parameters: initial oven temperature was 40 °C kept for 4 min, then raised at 5 °C/min to 100 °C followed by 10 °C/min to 220 °C, and kept for 7 min at 220 °C. Samples were injected by means of the headspace sampler in splitless mode (2 min). Injection port temperature was kept at 220 °C, pressure was 10 psi, and carrier gas (helium) flow was 2.2 mL/min. Detector temperature was 260 °C. A headspace sampler was equipped with a standard 1 mL loop. Carrier gas pressure was 14.9 psi, vial pressure was 4.4 psi, and injection time was 0.50 min. Samples were heated for 30 min at 50 °C.

Qualitative analysis was done by comparison of retention times of standards and corresponding peaks in beer samples. For method evaluation peak areas have been measured and expressed in the integrator units (pA·s). In beer sample analysis the concentrations of the compounds of interest have been determined according to an internal standard method with 1-pentanol as a standard and results are expressed in mg/L.

SPME Headspace Analysis. A Supelco SPME fiber holder (manual) and an 85 μm polyacrylate (PA)-coated fiber were used for the SPME method. Before use, the fiber was preconditioned in the GC injection port at 300 °C for 2 h. Sampling parameters were a subject of investigation, and the analysis conditions given herein have been established as the optimal parameters. When samples were analyzed using the SPME method, a 0.900 mL single-taper liner used in the headspace method was replaced by a narrow 1.5 mm i.d. direct liner (Yang and Peppard, 1994). The same chromatographic parameters were used as in the SHS method except the injection port temperature, which was 240 °C. SPME fiber was desorbed for 5 min. Samples were heated in a water bath at 50 °C for 60 min.

RESULTS

As a reference method for SPME, an SHS method described by Diedericks (1996), was utilized. Column type has been changed and separation parameters have been modified to avoid the necessity of oven cooling. Peaks of 3-methyl-1-butanol and 2-methyl-1-butanol have not been resolved under these circumstances. However, separation of these compounds plays an insignificant role in routine control of the aroma-forming constituents, as the odor thresholds are very similar for both of them (70 and 65 mg/L, respectively) (Meilgaard, 1982).

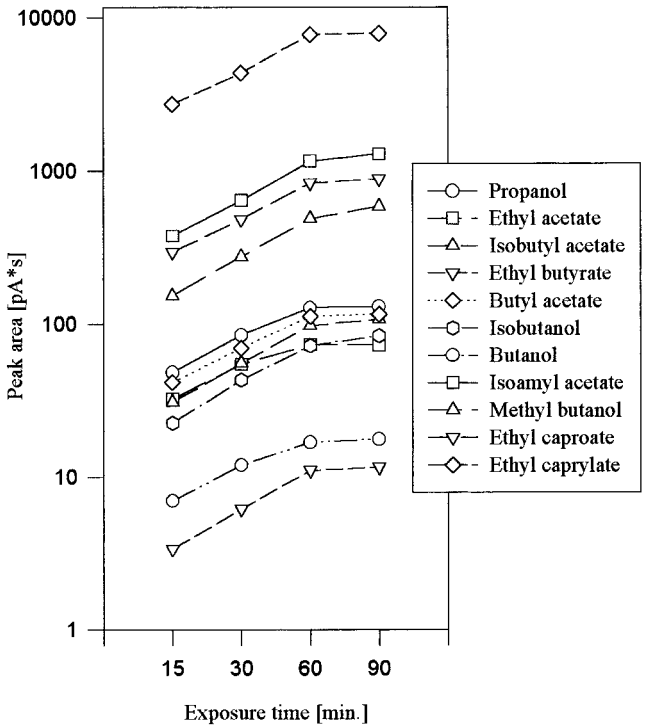

Figure 1. SPME adsorption time profile determined for the standard mixture of alcohols and esters. Peak areas are projected on a logarithmic scale. Analyzed compound concentrations are reported under Experimental Procedures.

SPME Analysis. Of the commercially available fiber coatings probably the most thoroughly investigated are poly(dimethylsiloxane) (PDMS) and polyacrylate (PA) ones. It has been established that PA fiber is more suitable for the analysis of more polar compounds, whereas PDMS is recommended for the nonpolar constituents (Steffen and Pawliszyn, 1996). Results described herein have been obtained using the PA fiber.

Optimization of Desorption Time. Temperature of desorption should be high enough to release volatiles adsorbed on a fiber totally, as the analyte carry over influences the quantitation and requires additional desorptions before the next sampling. The limiting factor is the compound character and the fiber resistance to high temperature. Compounds from the fiber have been desorbed in 240 °C for 1, 3, and 5 min. After the first desorption, fiber was desorbed for a second time to check whether the process was complete. One minute of desorption was revealed to be incomplete, whereas no compounds were present when the fiber was reinjected after 3 and 5 min desorptions. As the repeatability was highest for 5 min of desorption time, this parameter has been chosen for the subsequent analyses.

Optimization of Exposure Temperature and Time. Preparation of samples in SHS methods often involves sample heating. Beer for direct headspace analysis is usually heated to 40−60 °C, as the temperature influences the vapor pressure of analytes and this is therefore a simple way of improving the sensitivity of headspace methods. SPME analysis has been performed at 50 °C, similarly to SHS analysis. Heating the sample to this temperature resulted in 2−3 times bigger peak areas compared to the analyses performed at 30 °C.

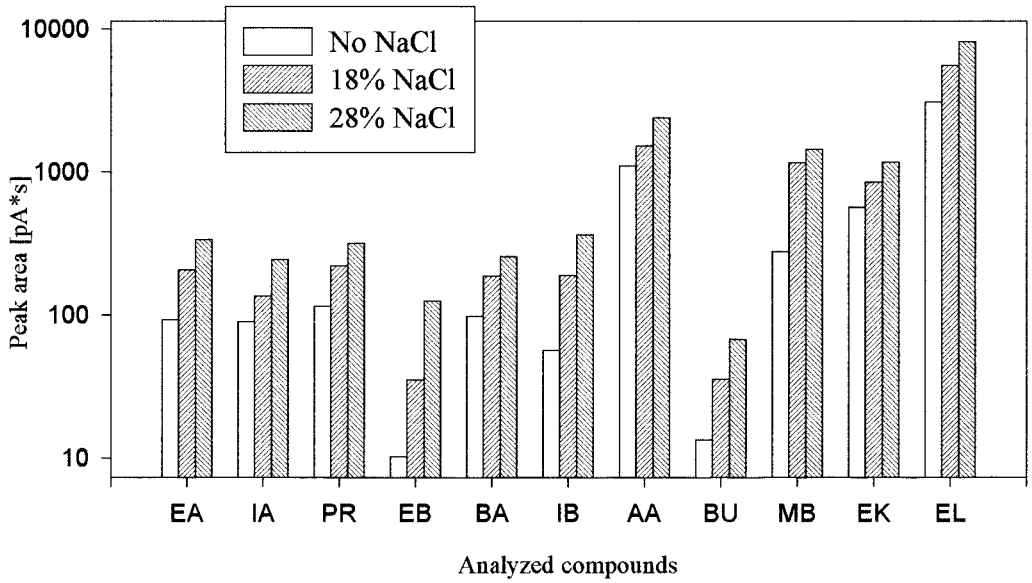

Figure 2. Peak areas determined for different salt solutions of investigated alcohols and esters: EA, ethyl acetate; IA, isobutyl acetate; PR, propanol; EB, ethyl butyrate; BA, butyl acetate; IB, isobutanol; AA, isoamyl acetate; BU, butanol; MB, methyl-1-butanol; EK, ethyl caproate; EL, ethyl caprylate.

Table 1. Comparison of Repeatability, Linearity, and Limits of Detection of SHS and SPME Methods Used for Beer Alcohol and Ester Determination

		SHS			SPME		
compound	calibration curve range (mg/L)	LOD[a] (mg/L)	r^b	RSD[c] (%)	LOD (mg/L)	r	RSD (%)
ethyl acetate	0.29−52.96	0.015	0.9999	1.79	0.026	0.9999	2.11
isobutyl acetate	0.05−10.12	0.010	0.9997	2.12	0.008	0.9993	1.80
propanol	0.28−56.04	0.020	0.9999	2.26	0.020	0.9999	3.76
ethyl butyrate	0.04−8.64	0.008	0.9979	4.19	0.004	0.9999	8.83
butyl acetate	0.04−8.64	0.010	0.9999	2.15	0.004	0.9998	3.55
isobutanol	0.30−59.92	0.020	0.9999	2.01	0.010	0.9999	7.10
isoamyl acetate	0.29−59.16	0.010	0.9999	1.78	0.004	0.9999	4.82
butanol	0.05−9.24	0.015	0.9997	3.87	0.005	0.9999	7.37
methyl-1-butanol	0.84−167.96	0.017	0.9997	1.34	0.003	0.9999	2.74
ethyl caproate	0.05−11.00	0.010	0.9999	2.28	0.002	0.9995	7.32
ethyl caprylate	0.08−16.20	0.012	0.9998	10.03	0.002	0.9981	10.80

[a] LOD, limit of detection. [b] r, regression coefficient of the standard curve. [c] RSD, relative standard deviation (estimated for peak areas, $n = 7$).

SPME is considered complete when the analyte concentration has reached equilibrium between the sample matrix and the fiber coating. The equilibration time is dependent on type of analyte, matrix, and extraction mode. Choosing the appropriate fiber coating will also influence the time required to reach equilibrium state. Standard samples in triplicates were incubated at 50 °C for 15, 30, 60, and 90 min with the fiber exposed in the headspace. The vials have not been shaken nor their contents stirred. The results are shown in Figure 1. According to the literature data PA fiber usually requires longer extraction times compared with other fibers (Pawliszyn, 1997; Steffen and Pawliszyn, 1996). In this study the increase of peak areas was least for most of the compounds after 60 min. For the quantitation of analytes no equilibrium state is required, assuming that the standard and sample preparations are carried out under the same conditions, so the subsequent analyses were performed using 60 min of exposure time. However, satisfactory results, with no significant decrease in sensitivity or repeatability, were also obtained with 30 min of exposure time, which remarkably shortens the sample preparation step.

Salt Effect. The behavior of selected flavor compounds in the presence of various salt concentrations in SPME adsorption was described by Yang and Peppard (1996). Depending on the compound type, their adsorption can increase with salt concentration increase, then level off or decrease, and finally decrease with higher salt concentration. For all analyzed alcohols and esters, peak areas increased with increasing salt concentration (Figure 2.). Addition of sodium chloride to analyzed beer lowers the detection limits of the SPME method and therefore may be helpful in running samples in which esters occurring in trace quantities are the main point of interest.

Comparison of SPME and SHS Methods. For methods comparison the following parameters have been determined: limits of detection, repeatability, and linearity. For SPME and SHS standard solutions of the same concentrations have been used (see Experimental Procedures). Results of this comparison are summarized in Table 1. For both methods five-point standard curves have been prepared, covering the concentrations of the compounds usually found in beers of Pilsner type. The regression coefficients for curves for all but one compound determined by using the two methods were >0.998. This shows that both methods are characterized by high linearity in the examined concentration ranges.

Limits of detection for both methods were determined

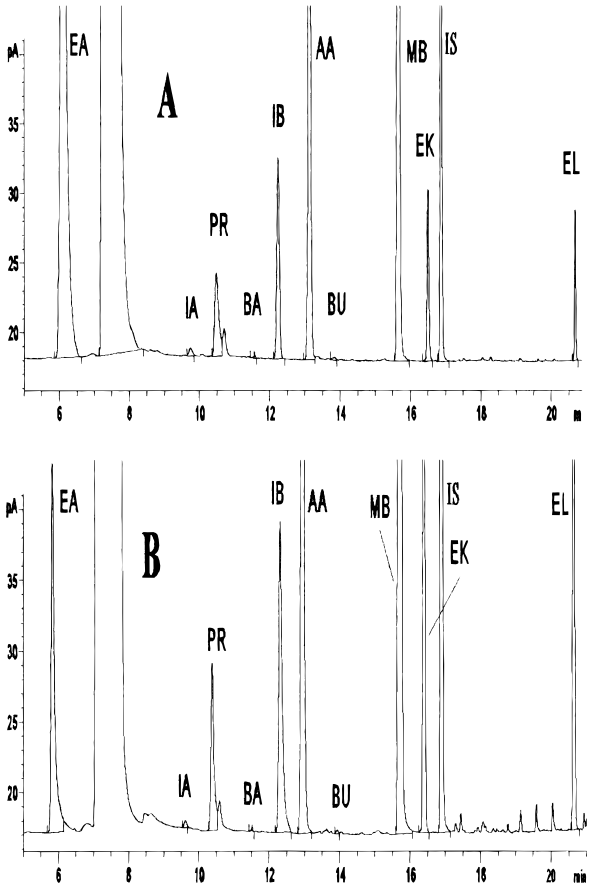

Figure 3. Chromatograms of Pilsner type beer alcohols and esters determined by SHS (A) and SPME (B) methods: IS, internal standard; other abbreviations as in Figure 2.

by extrapolation of the lowest concentration points of the standard curves. For ethyl acetate the limit of detection determined with the SHS method was lower than that found with the SPME method; for propanol the limits of detection were the same for both methods, whereas for the remaining compounds the limits of detection determined with the SPME method were lower than those determined with the SHS method, usually 2−5 times lower (Table 1).

Repeatability of the SHS method determined by peak area comparison was better than that of the SPME method, though one must remember that the former was automated, whereas a manual version of the SPME holder was used. Except for two compounds, ethyl butyrate and ethyl caprylate, the RSD for the SHS method was <4%, while the RSD values for SPME exceeded 4% for 6 of 11 compounds (Table 1).

Both methods have been applied for the analysis of beer samples. Three bottles of Pilsner type beer purchased in a local store were analyzed. Figure 3 shows typical chromatograms obtained by SHS and SPME methods. In none of the analyzed beers was ethyl butyrate detected. The concentrations of the analyzed compounds in one beer sample are shown in Table 2. Apart from dominating isoamyl alcohols, four compounds were present in concentrations >1 mg/L: ethyl acetate, propanol, isobutanol, and isoamyl acetate. For compound quantitation the internal standard method has been chosen, and for the SHS method RSD values for five compounds were <1%; for two compounds, present in low concentrations, the RSD values approached 10%. For the SPME method RSD values

Table 2. Concentration of Dominating Higher Alcohols and Esters in Pilsner Type Beer Determined by Using SHS and SPME Methods

	SHS		SPME	
compound	$\bar{x}^a$ (mg/L)	RSD^b (%)	$\bar{x}$ (mg/L)	RSD (%)
ethyl acetate	11.40	1.06	11.95	5.48
isobutyl acetate	0.19	8.83	0.13	4.33
propanol	2.98	0.89	3.03	3.23
butyl acetate	0.15	0.39	0.11	0.31
isobutanol	6.55	0.62	7.12	0.93
isoamyl acetate	2.79	0.56	2.03	3.01
butanol	0.17	10.18	0.18	6.80
methyl-1-butanol	49.42	0.32	53.37	1.03
ethyl caproate	0.35	1.26	0.31	1.84
ethyl caprylate	0.44	1.32	0.37	3.15

[a] Results shown are mean of three replicates. [b] Relative standard deviation.

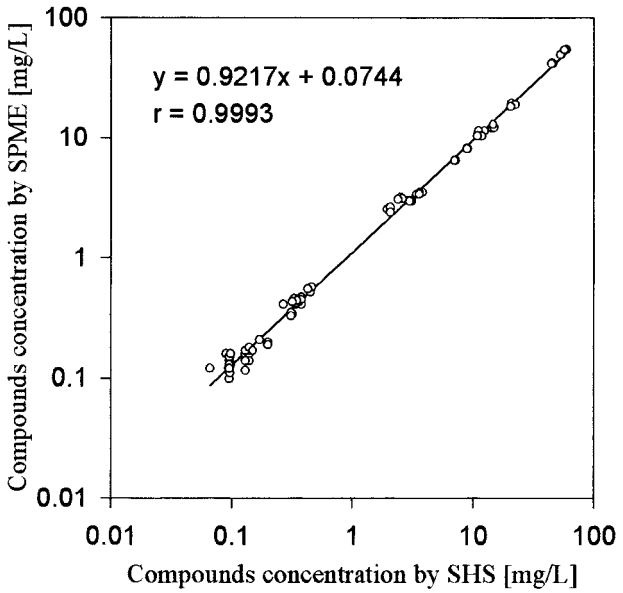

Figure 4. Linear regression for alcohols and esters in beer samples determined by SHS and SPME methods. Results shown are for 10 compounds measured in 3 Pilsner type beers in triplicate ($n = 90$).

estimated for compound concentrations were satisfactory, being <7%. Alcohol and ester contents determined by using the SPME method were somewhat higher than those determined by SHS (Table 2.). Results obtained by using these two methods are highly correlated, as presented in Figure 4.

DISCUSSION

The presented data indicate a good agreement between SHS and SPME determinations of alcohols and esters in beer. SPME can be a relatively cheap alternative to the automated SHS, with potential perspectives of method parameters improvement (sensitivity, exposure time) due to a variety of fiber coatings developed.

The results show the performance of both methods in the analysis of beer constituents present in concentrations ranging usually from high parts per billion up to 10−150 ppm. There are a number of ways to improve SHS and SPME method sensitivity: changing headspace/sample volume or adding salts, to name only two. However, the concentrations of alcohols and esters present in beer do not require detection limits enhancing ones established for a non-salted-out solutions. By a proper selection of the SPME fiber certain selectivity

and different sensitivity can be achieved. Recently released on the market 75 μm PDMS/Carboxen fiber (Supelco 5-7318) has also been examined. Detection limits using this fiber were much lower than for PA or headspace methods, and the peak areas observed were from 3 (isobutanol) to 80 (ethyl butyrate) times higher than for PA-coated fiber. The PDMS/Carboxen fiber has been used for the determination of low concentrations of BTEX and other volatiles in water characterized by very low detection limits and a good linearity within the parts per billion concentration range (Popp and Paschke, 1997). However, higher alcohols and esters are present in beer in relatively high concentrations. For example, 3-methylbutanol can reach 160 ppm in stouts; isobutanol, usually present in Pilsner type beer in concentrations of 5−12 ppm, can reach 84 or 98 ppm in wheat beer and stout, respectively. Ethyl acetate, found in American ales in concentrations up to 15 ppm, can reach 68 ppm in Belgian top fermented beers (Suomalainen and Nykkanen, 1983). Therefore, linearity rather than ultrahigh sensitivity is of primary concern in the analysis of these compounds. Using the PDMS/Carboxen fiber in the determination of beer alcohols and esters has shown that in samples prepared as for SHS analyses the fiber responded nonlinearly in higher concentrations of some analytes where PA response was still linear, probably due to a saturation of the Carboxen/PDMS fiber. Shortening the sampling time to 2 min (in 30 °C) resulted in lower absorption in the fiber, and the linearity improved. This example confirms the potential application of other fiber types in beer analysis.

Quantitation based on an internal standard is a technique used in SHS methods (Diedericks, 1996; Drawert, 1982) and has been reliable for SPME analyses. However, in the preparation of standard curves it is virtually impossible to generate a matrix identical to beer but free of alcohols and esters. The use of water instead may be a possible source of the differences in the results obtained using SHS and SPME methods (Table 2), as the fiber can perform differently in these matrices.

The results presented in this paper indicate that analyses of esters and alcohols by SPME should be a reliable method for the determination of other beer compounds of this type, which not have been a subject of the presented study.

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