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Preparation of novel solid-phase microextraction fibers by sol–gel technology for headspace solid-phase microextraction-gas chromatographic analysis of aroma compounds in beer

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Abstract

3-(Trimethoxysilyl)propyl methacrylate (TMSPMA) was first used as precursor as well as selective stationary phase to prepare the sol–gel-derived TMSPMA-hydroxyl-terminated silicone oil (TMSPMA-OH-TSO) solid-phase mircroextraction (SPME) fibers for the analysis of aroma compounds in beer. TMSPMA-OH-TSO was a medium polarity coating, and was found to be very effective in carrying out simultaneous extraction of both polar alcohols and fatty acids and nonpolar esters in beer. The extraction temperature, extraction time, and ionic strength of the sample matrix were modified to allow for maximum sorption of the analytes onto the fiber. Desorption temperature and time were optimized to avoid the carryover effects. To check the matrix effects, several different matrices, including distilled water, 4% ethanol/water (v/v) solution, a concentrated synthetic beer, a "volatile-free" beer and a real beer were investigated. Matrix effects were compensated for by using 4-methyl-2-pentanol as internal standard and selecting the "volatile-free" beer as working standard. The method proposed in this study showed satisfactory linearity, precision and detection limits and accuracy. The established headspace SPME-gas chromatography (GC) method was then used for determination of volatile compounds in four beer varieties. The recoveries obtained ranged from 92.8 to 105.8%. The relative standard deviations (RSD, $n = 5$) for all analytes were below 10%. The major aroma contributing substances of each variety were identified via aroma indexes.

Keywords: Sol–gel; 3-(Trimethoxysilyl)propyl methacrylate; Solid-phase microextraction; Beer; Volatile compounds

1. Introduction

Aroma substances are very important in beer as they make a major contribution to quality of the final product. A great number of volatile compounds, belonging to very heterogeneous groups such as alcohols, esters, organic acids, aldehydes, ketones, terpenes, sulfur compounds, amines, phenols etc., have been identified in beer, and the different substances may influence the beer aroma and flavour to a very different degree. Some volatiles are of great importance, and may contribute greatly to the beer flavour, while others are important merely in building up the background flavour of the product. A better understanding of the key aroma compounds would be of significant importance, as this information is valuable for the modern brewing technology, particularly in the selection of raw materials and yeast strain, beer quality control and product development.

Commonly, direct injection is not suitable for beer analysis. When beers are analyzed by direct injection, due to their high content in sugar and to the high temperature in the injector and in the column, the caramelization of sugars is possible, causing irreversible damage to the column, especially capillary column. Additionally, the injection of beer samples produces a large amount of particles that can plug column tips causing variation in carrier fluxes and peak shapes. Therefore, the removal of non-volatile components is a prerequisite for gas chromatographic (GC) analysis. Moreover, the analysis

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of the minor volatile compounds with direct injection is quite difficult due to their very low concentration level. In these cases, the sample pretreatment and concentration method was thus very important for the gas chromatographic analysis of volatile compounds in beer.

Several extraction–concentration methods have been employed for the analysis of volatile compounds in beers, such as liquid–liquid extraction [1], simultaneous extraction and distillation [2], solid-phase extraction [3], supercritical fluid extraction [4], etc. Most of these methods produce extracts with a flavor composition that is representative of the liquid matrix and not of the headspace. Chromatographic signals of trace substances may be obscured by high concentrations of low-volatile compounds. Another shortcoming of these methods is that the extracts have to be concentrated prior to analysis, resulting in losses of low-boiling volatiles. Headspace analysis can overcome these disadvantages, allowing analysis of the volatile fraction only. The most widely used headspace sampling technique for volatile isolation is, however, static, dynamic headspace analysis or purge and trap technique. Its main advantage is that no sample cleanup is necessary prior to GC analysis. However, special instrumentation coupled to the gas chromatograph is required to trap the volatiles, and often, the sensitivity of the method is low. These drawbacks can be overcome by using headspace solid-phase microextraction (HS-SPME) technique. It is a simple, fast, sensitive and solvent-free extraction technique that enables the extraction and the concentration steps to be performed simultaneously.

Due to these positive attributes, HS-SPME has been successfully used in beer samples. The use of SPME in beer analysis mainly focused on analysis of the off-flavours, such as sulfur compounds [5,6] and carbonyl compounds [7]. Recently, Steinhaus et al. [8] applied SPME in combination with stable isotope dilution assay (SIDA) for the analysis of the hoppy aroma substance linalool in beer.

Despite rapid advancement in the area of SPME application, a number of important problems still remain to be solved. First, existing SPME coatings are designed to extract either polar or nonpolar analytes from a given matrix. Such SPME fibers are not very convenient for beer samples where analytes from different chemical classes representing a wide polarity range are present and all need to be analyzed. Second, the determination of some of the ultra-trace flavour compounds in beers is challenging due to the low sensitivity of some of the existing SPME coating. Increasing the coating thickness is an effective way of enhancing surface area and sample capacity. In addition, preparing a porous coating is another route to enhance extraction efficiency. However, thick coating is difficult to immobilize on fused silica surface merely by conventional approaches [9], such as immobilizing the coating using a high-temperature epoxy resin. Third, thermal and solvent restrictions are encountered with traditional SPME fibers because the majority of these fibers are prepared by mere physical deposition of the polymer coating on the substrate of the fused-silica fiber [10].

Sol–gel coating technology [11–13], established by Malik and co-workers, has solved most of these problems. It has been used to create surface-bonded SPME coating both on the outer surface of the fused-silica fiber (fiber-based SPME) [14,15] and on the inner surface of a capillary (in-tube SPME or capillary microextraction (CMC)) [16,17]. In our group, hydroxyl-crown ether [18,19] and calixarene [20,21] coated fibers had been prepared with this technique. Moreover, the combination of sol–gel approach and cross-linking technique for the preparation of SPME fibers had also been reported by us including poly (methylphenylvinylsiloxane) (PMPVS) [22], open crown ether [23], and silicone/DVB [24] coatings. Compared with conventional SPME fibers, they showed better selectivity and sensitivity toward polar, nonpolar and high-boiling aromatic compounds such as phenols [18], aromatic amines [19], benzene derivatives [20], PAHs [22] and phthalates [23]. In this paper, our interest is to develop a novel fiber for solid-phase microextraction of both polar and nonpolar aliphatic compounds.

3-(Trimethoxysilyl)propyl methacrylate (TMSPMA), which served as a bifunctional reagent, contains both methacrylate and alkoxysilane groups. It has been widely used as a coupling agent in the preparation of organically modified silicate materials [25] and stationary phases [26], etc. To date, we are not aware of any report on the application of TMSPMA as SPME coating. In this work, a new SPME coating made from TMSPMA and hydroxyl-terminated silicone oil (OH-TSO) was developed by sol–gel and free radical polymerization and was applied for the simultaneous extraction of both polar alcohols and fatty acids and nonpolar esters. Several extraction variables and desorption conditions were optimized. Moreover, the matrix effects on the extraction were investigated in detail. An accurate quantitative method to remove the matrix interference was developed for the determination of volatile compounds in four beer varieties. The major aroma contributing substances of each variety, which can provide valuable information for modern brewing technology, particularly in the quality control, were also identified via aroma indexes.

2. Experimental

2.1. Instrumentation

To mix various solution ingredients thoroughly, an Ultrasonator model KQ-50DE (Kunsan Ultrasonator Instrument Corporation, Kunsan, China) was employed. A Centrifuge model TGL-16C (Shanghai Anting Instrument Factory, Shanghai, China) was used to separate the sol solution from the precipitate. The fused-silica fiber (120 μm, o.d.) with protective polyimide coating was provided by the Academy of Post and Telecommunication, Wuhan, China. A magnetic stirrer DF-101B (Leqing, China) was employed for stirring the sample during extraction.

A pH meter model pHS-2C (Shanghai Hongyi Instrument Corporation, Shanghai, China) was used to prepare standard solutions. A homemade SPME syringe with sol–gel-derived TMSPMA-OH-TSO fiber was used to transfer the extracted sample to the GC injector for analysis. The SPME holder, for manual sampling, and different commercially available fibers: polydimethylsiloxane (PDMS, 100 μm), polydimethylsiloxane–divinylbenzene (PDMS–DVB, 65 μm) and polyacrylate (PA, 85 μm), were purchased from Supelco (Bellefonte, PA, USA). Prior to use, all the fibers were conditioned following the manufacturer's recommendations.

SPME-GC experiments were carried out on a GC-2000 gas chromatograph (Shandong Lunan Ruihong Chemical Instrument Corporation, Shandong, China) equipped with a flame ionization detector, on a laboratory-made PEG20M coated fused silica capillary column (35 m × 0.32 mm i.d.). Online data collection and processing was done on Chromatopac model SISC-SPS (The Scientific Instrument Software Company, Beijing, China). The GC oven temperature was programmed from 40 °C (held for 8 min) to 230 °C at 5 °C/min with a 20 min hold at the final temperature. The injection port temperature was 300 °C and the detector temperature was 300 °C. The injection was made in the splitless mode. Nitrogen was used as the carrier gas at a constant flow rate of 0.3 ml/min.

2.2. Reagents and materials

OH-TSO was purchased from Chengdu Center for Applied Research of Silicone (Chengdu, China). Tetraethoxysilane (TEOS) and poly(methylhydrosiloxane) (PMHS) were obtained from the chemical plant of Wuhan University (Wuhan, China). TMSPMA was obtained from Huachang Academy of applied techonology (Wuhan, China). Trifluoroacetic acid (TFA) was purchased from Merck, Germany.

The following alcohols, esters and fatty acids were studied: 1-propanol, isobutanol, isoamyl alcohol, 1-hexanol, linalool, β-phenylethanol; ethyl acetate, isobutyl acetate, ethyl butyrate, isoamyl acetate, ethyl hexanoate, ethyl lactate, ethyl octanoate, ethyl decanoate, diethyl succinate; acetic acid, hexanoic acid, octanoic acid and decanoic acid. 4-methyl-2-pentanol was used as internal standard. These standards, with purity above 99%, were supplied by Aldrich (Steinheim, Germany), Sigma (St. Louis, MO, USA), Shanghai Organic Reagent Plant (Shanghai, China), Beijing Chemical Plant (Beijing, China) and Tianjing Chemical Plant (Tinjing, China).

Beer samples were purchased from four China breweries: Ref "Beer 1" (4.0%, v/v, ethanol; 11 °P, original wort concentration), Ref "Beer 2" (3.1%, m/m, ethanol; 10 °P, original wort concentration), Ref "Beer 3" (4.0%, v/v, ethanol; 11 °P, original wort concentration), Ref "Beer 4" (5.2%, v/v, ethanol; 11 °P, original wort concentration). All these beers were produced in April 2004.

2.3. Fiber preparation

Prior to sol–gel coating, the 6-cm-long fused-silica fiber was dipped in acetone for 3 h to remove the protective polyimide layer, in a 1 M NaOH solution for 1 h to expose the maximum number of silanol groups on the surface, cleaned with water, and dipped in 0.1 M HC1 solution for 30 min to neutralize the excess NaOH, cleaned again with water and air-dried at room temperature.

Briefly, a sol solution was prepared by dissolving 90 mg of OH-TSO, 100 μl of TEOS, 50 μl of TMSPMA, 10 mg of PMHS, 8 mg of benzophenone (BP) and 80 μl of TFA (5% H_2O) in 100 μl of methylene chloride. The mixture was then mixed thoroughly by ultrasonic agitation (5 min), centrifuged at 12,000 rpm (8 min) and the clear supernatant of the sol solution was transferred to another clean vial for fiber coating. The treated fiber was inserted vertically into the sol solution and held for about 30 min, and then the fiber was drawn out from the sol solution, during which a sol–gel coating was formed on the outer surface of the fiber end (about 1 cm). The coating process was repeated several times in the same sol solution until the desired thickness of the coating was obtained. After that the fibers were irradiated under ultraviolet light for 30 min, then placed in a desiccator for 12 h at room temperature and conditioned at 300 °C under nitrogen protection for 2 h in the GC injection port. A OH-TSO fiber was also coated for comparison by sol–gel technique with an identical preparation procedure except that TMSPMA was not added.

2.4. Preparation of standard solutions and working standards

A standard solution containing all the analytes was prepared in ethanol at a concentration of each 1 mg/1 and used for direct injection. A global standard solution of 1-propanol (3.20 mg/ml), isobutanol (4.80 mg/ml), isoamyl alcohol (14.58 mg/ml), 1-hexanol (0.41mg/ml), linalool (0.0348 mg/ml), β-phenylethanol (3.06 mg/ml), ethyl acetate (4.05 mg/ml), isobutyl acetate (0.017 mg/ml), ethyl butyrate (0.0528 mg/ml), isoamyl acetate (0.087 mg/ml), ethyl hexanoate(0.034 mg/ml), ethyl lactate (14.0 mg/ml), ethyl octanoate (0.051 mg/ml), ethyl decanoate (0.06 mg/ml), diethyl succinate (4.16 mg/ml), acetic acid (15.75 mg/ml), hexanoic acid (0.186 mg/ml), octanoic acid (0.273 mg/ml) and decanoic acid (0.09 mg/ml) was prepared in ethanol for HS-SPME. A standard solution of internal standard was prepared in ethanol to yield 12.01 mg/ml of 4-methyl-2-pentanol.

A concentrated synthetic beer was prepared by dissolving 11 g L(+)-tartaric acid, 40 ml of ethanol and a suitable amount of sodium hydroxide in deionized water to give 1 l of solution. The percent of ethanol and pH value of the synthetic beer were 4% (v/v) and 4.5, respectively, which reproduced the properties of Beer 1 studied.

To generate a matrix identical to the real beer samples but free of volatile alcohols, fatty acid and esters, a "volatile-

free" beer was prepared as follows: 250 ml of Beer 1 was distilled under vacuum to remove ethanol and other volatile components, and then cooled, filtrated, 10 ml ethanol added to the volumetric flask, and diluted with deionized water to the scale. The non-volatile components remain unchanged, and the concentration of the ethanol (4%, v/v) and pH value (4.5) of the "volatile-free" beer was equal to that of Beer 1 studied.

All of the stock solutions and the working standards were stored at 4 °C.

2.5. HS-SPME procedure

To avoid any direct contact with the sample matrix, HS-SPME was performed in this work. For each SPME analysis, 5 ml of "volatile-free" beer was placed into a 10-ml glass vial with 2 g of NaCl and a little magnetic stir bar. A 20 μl of global stock standard solution and a 5 μl of standard solution of 4-methyl-2-pentanol (internal standard) were added to the sample. Then, the vial was tightly capped with a butyl rubber stopper wrapped with PTFE sealing tape and an aluminum cap. Afterward, the stainless steel needle, where the fiber is housed, was pushed through the vial septum, and then the fiber was pushed out of the housing and exposed to the headspace above the sample for 30 min at 40 °C. After extraction, the fiber was removed from the sample vial and immediately inserted into the heated injector of the gas chromatograph (300 °C) with 5 min desorption time. Blank runs were completed at least once daily before sampling to ensure no carryover of analytes from previous extractions.

2.6. Beer analysis

The samples of cans of beer were cooled to 4 °C to minimize the loss of very volatile compounds. The container was opened and 5 ml of beer sample was pipetted into a 10-ml glass vial containing 2 g of NaCl and a little magnetic stir bar.

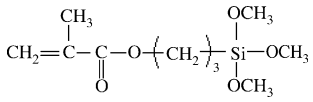

Fig. 1. The structure of TMSPMA.

A 5 μl of internal standard solution of 4-methyl-2-pentanol was added, giving final concentrations of 12.01 μg/ml. The vials were tightly capped. SPME experiments were performed under the same conditions as standard solutions. Each analysis was undertaken in quintuplicate using different vials.

3. Results and discussion

3.1. Characteristics of TMSPMA-OH-TSO fiber

Unlike the common sol–gel process, in which only one metal alkoxide is used as the precursor to produce silica fiber, our process involves two different silica monomers (TEOS and TMSPMA) as co-precursors. The use of TMSPMA as the co-precursor can provide important advantages. It can not only serve as a cross-linking agent but also act as selective stationary phase in the sol–gel coating due to its special structure (Fig. 1). When TMSPMA is introduced in the sol solution, two sets of chemical reactions can occur simultaneously. First, the trimethoxysilyl groups in the monomer can be hydrolyzed to silanol groups, which are allowed to react with other sol–gel active components, resulting in chemical bonding of TMSPMA to the evolving sol–gel network. Second, the vinyl substituent in the monomer can undergo free radical polymerization reactions under ultraviolet with benzophenone as an initiator. A simplified scheme of the sol–gel TMSPMA-OH-TSO coating on the fused-silica fiber surface is presented in Fig. 2.

Fig. 3 shows the IR spectra of sol–gel-derived OH-TSO, TMSPMA-OH-TSO stationary phases and pure TMSPMA.

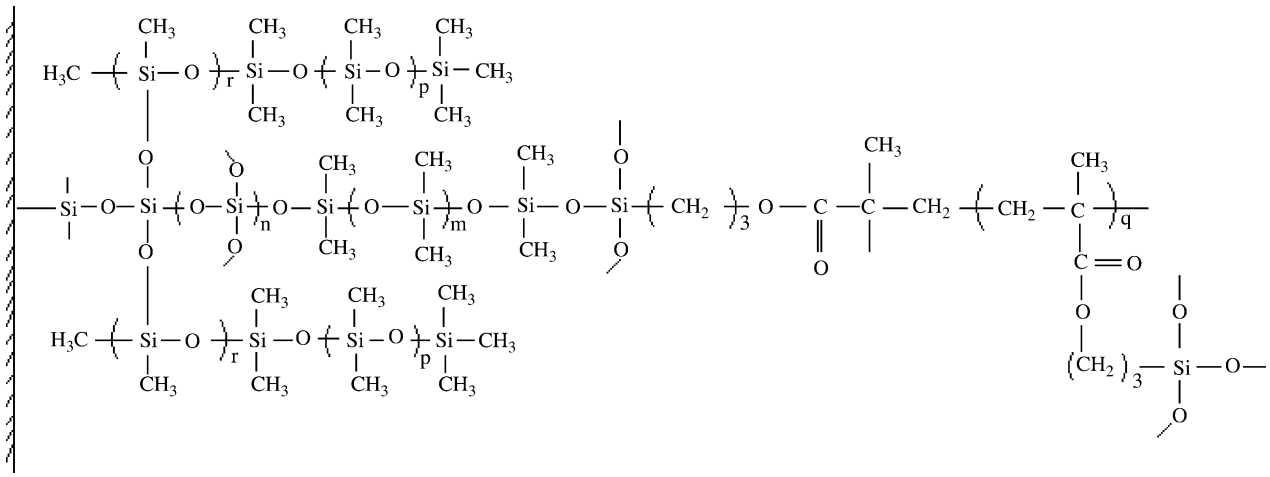

Fig. 2. A simplified structure of the sol–gel-derived TMSPMA-OH-TSO polymeric coating.

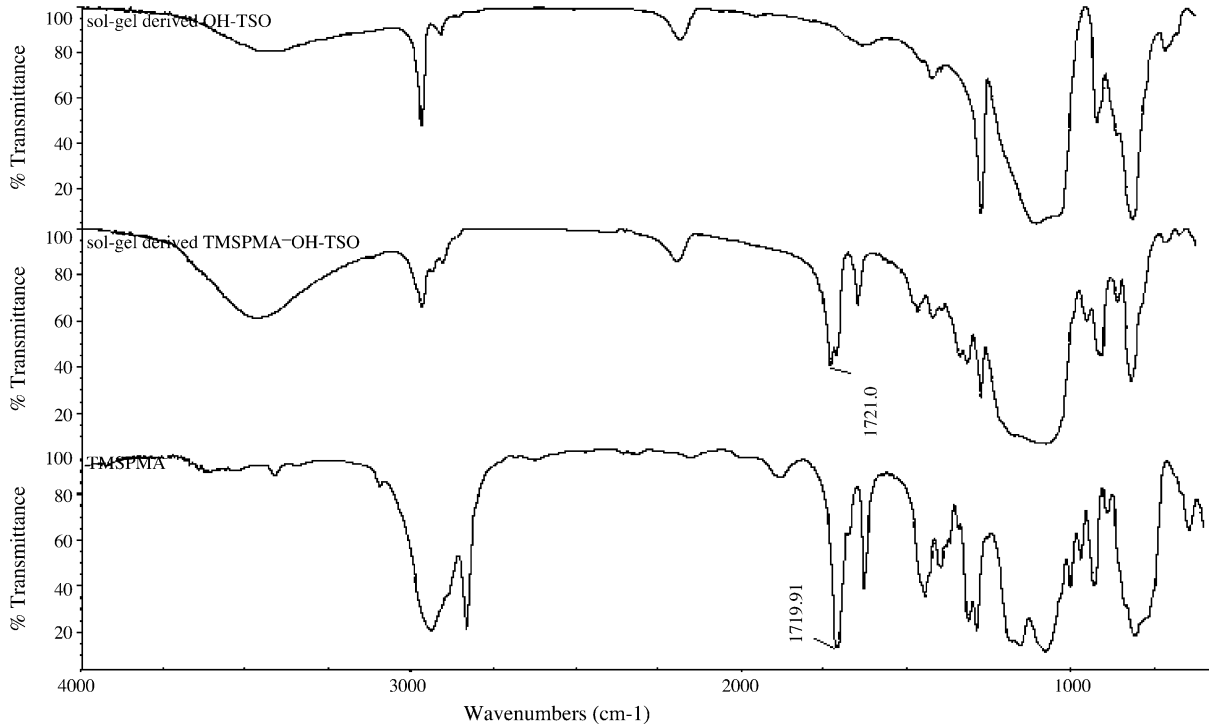

Fig. 3. IR spectra of sol–gel derived OH-TSO coating (top), sol–gel derived TMSPMA-OH-TSO coating (middle) and pure TMSPMA (bottom).

The feature identified by TMSPMA (1719.91 cm^{-1} ($\nu_{C=O}$)) also appeared in the sol–gel-derived TMSPMA-OH-TSO coating. It proved the successful binding of TMSPMA to the stationary phase.

Fig. 4 shows the extraction capability of the sol–gel coated OH-TSO fiber and TMSPMA-OH-TSO fiber with the identical preparation procedure. Owing to the special functional group (–COO–) in TMSPMA, TMSPMA-OH-TSO fiber gave much higher response to both the polar alcohols and fatty acids and nonpolar esters than the OH-TSO fiber. Undoubtedly, TMSPMA plays an important role in the extraction.

Fig. 5 compares the extraction efficiencies of sol–gel-derived TMSPMA-OH-TSO fiber with commercial PDMS, PDMS-DVB and PA fibers. According to the principal of "like dissolves like", the polar analytes have higher affinity for polar coating. Considering the special structure and polarity of TMSPMA and PA, better adsorption efficiencies for polar alcohols and fatty acids were observed on PA and the sol–gel-derived TMSPMA-OH-TSO fibers, while the TMSPMA-OH-TSO fiber is more suitable for the analysis of these polar compounds because the sol–gel process provides a three-dimensional network leading to the enhanced surface area and sample capacity. At the same time, TMSPMA-OH-TSO shows much higher responses to nonpolar esters than PDMS and PDMS-DVB fibers also thanks to the outstanding material properties of sol–gel coating. As revealed from the figure, the sol–gel-derived TMSPMA-OH-TSO fiber is very convenient for the simultaneous extraction of both polar alcohols and fatty acids and nonpolar esters.

3.2. Optimization of HS-SPME process

Fig. 6 represents the extraction temperature profile for the volatile compounds in the "volatile-free" beer matrix. The optimum temperature for the extraction of volatile alcohols was

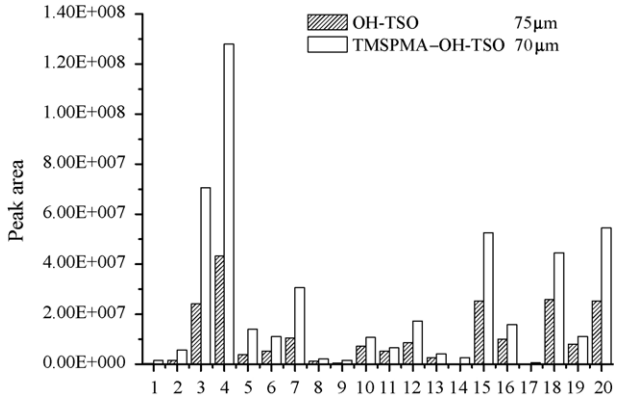

Fig. 4. Comparison of the extraction capability of the sol–gel coated OH-TSO and TMSPMA-OH-TSO fibers with the same preparation procedure. GC analysis conditions: 35 m × 0.32 mm i.d. laboratory-made PEG column; splitless injection; injector temperature, 300 °C; GC oven temperature, programmed from 40°C (hold for 8 min) to 230 °C (hold for 20 min) at a rate of 5 °C/min; nitrogen carrier gas; detector temperature 300 °C. SPME conditions: extraction time, 30 min; extraction temperature, 40 °C; saturated out with NaCl; magnetic stirring; desorption time, 5 min. Peaks: (l) 1-propanol; (2) isobutanol; (3) 4-methyl-2-pentanol; (4) isoamyl alcohol; (5) 1-hexanol; (6) linalool; (7) β-phenylethanol; (8) acetic acid; (9) hexanoic acid; (10) octanoic acid; (11) decanoic acid; (12) ethyl acetate; (13) isobutyl acetate; (14) ethyl butyrate; (15) isoamyl acetate; (16) ethyl hexanoate; (17) ethyl lactate; (18) ethyl octanoate; (19) ethyl decanoate; (20) diethyl succinate.

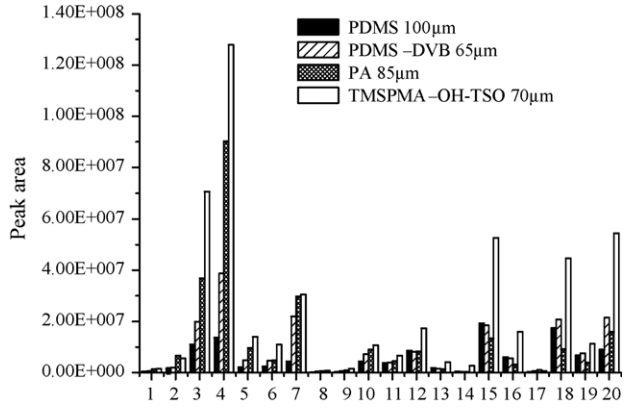

Fig. 5. Comparison of the extraction efficiency of the sol–gel-derived TMSPMA-OH-TSO fiber with commercial PDMS, PDMS-DVB and PA fibers. SPME-GC conditions are the same as in Fig. 4. Compounds notions are the same as in Fig. 4.

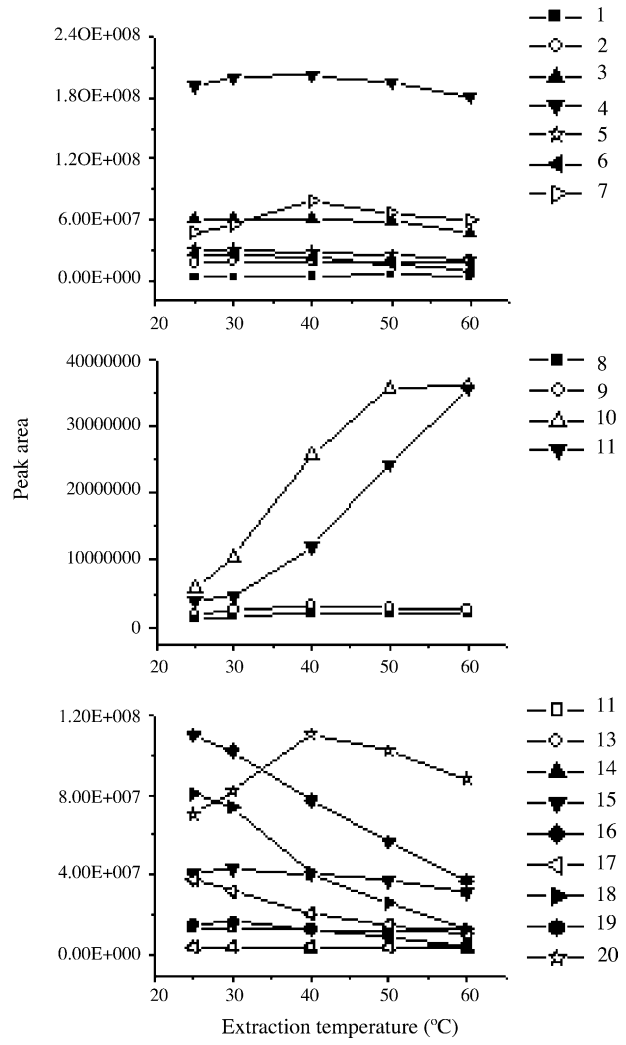

Fig. 6. The extraction temperature profile for the volatile compounds. SPME-GC conditions are the same as in Fig. 4 except for the extraction temperature. Compounds notions are the same as in Fig. 4.

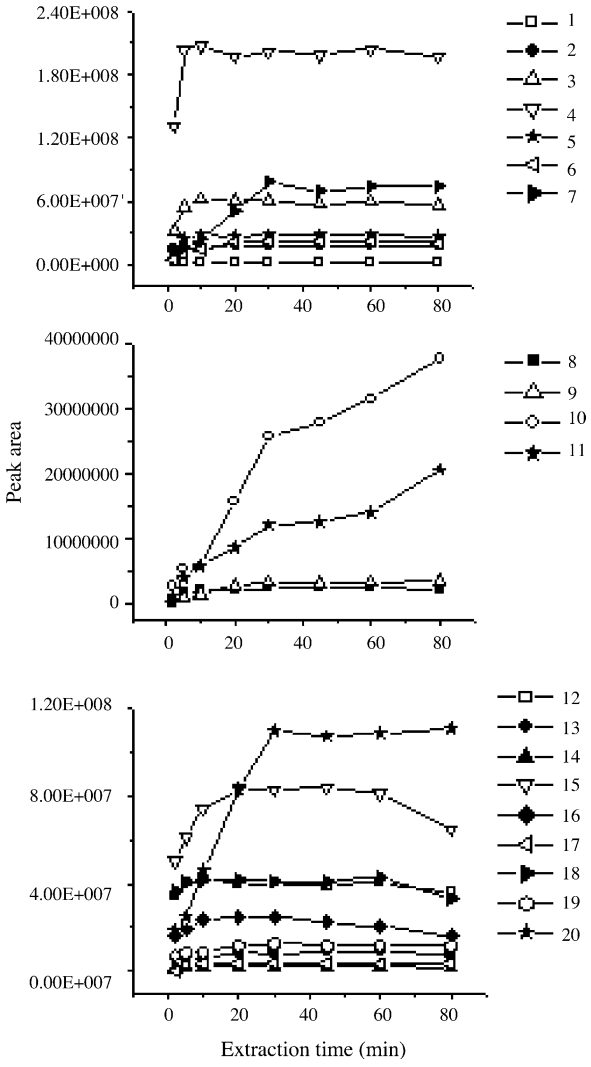

Fig. 7. The extraction time profile for the volatile compounds. SPME-GC conditions are the same as in Fig. 4 except for the extraction time. Compounds notions are the same as in Fig. 4.

40 °C. For the fatty acids, the extraction yield increased with an increase in temperature, while for the esters, it decreased except for diethyl succinate. Taking into account the quite low extraction efficiency for fatty acids at low temperature, 40 °C was selected as the optimum though a little extraction losses were found for esters.

Fig. 7 shows the extraction time profile for the volatile compounds in the "volatile-free" beer matrix. Ten minutes was enough for all alcohols to reach equilibrium except for linalool and β-phenylethanol, which reached equilibrium within 20 and 30 min, respectively. Twenty minutes was required for all esters to reach equilibrium with the exception of diethyl succinate, which reached equilibrium within 30 min. The adsorption equilibrium was reached for acetic and hexanoic acids within 30 min, while it was not attained for octanoic and decanoic acids even up to 80 min. The optimum extraction time was 30 min, which is sufficient to achieve

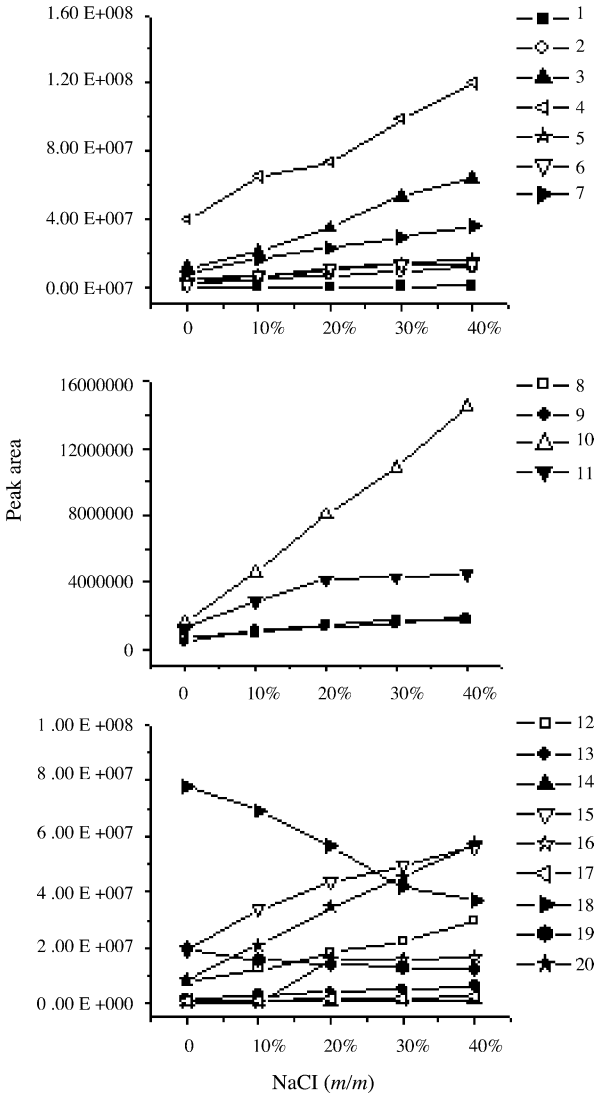

Fig. 8. The influence of ionic strength on the amount of volatile compounds extracted. SPME-GC conditions are the same as in Fig. 4, except for the content of sodium chloride. Compounds notions are the same as in Fig. 4.

the required sensitivity for fatty acids while does not suffer extraction losses for alcohols and esters.

The influence of the sodium chloride concentration in the "volatile-free" beer solution (from 0% (m/m) to saturation) on the extraction was studied (Fig. 8). With the exception of ethyl octanoate and ethyl decanoate, peak areas of most of the volatile compounds increased with the increase of salt concentration, attaining maxima when the solution was saturated. Thus, 2 g sodium chloride was added per 10-ml vial in the following experiments.

In order to investigate the carryover problems, four different desorption conditions were considered: 250 °C for 2 min, 250 °C for 5 min, 280 °C for 5 min and 300 °C for 5 min. No carryover was observed along all these experiments for the alcohols and esters. However, high percentages of carryover were found for the free fatty acids, as shown in Table 1. Effective release of the extracted polar analytes from the coatings

Table 1
The effect of desorption conditions on the carryover for free fatty acids

Desorption conditions	Carryover percentage (%)		
	Hexanoic acid	Octanoic acid	Decanoic acid
250 °C, 2 min	7.94	9.98	25.89
250 °C, 5 min	1.20	5.23	16.86
280 °C, 5 min	n.c.[a]	3.20	9.53
300 °C, 5 min	n.c.	n.c.	n.c.

[a] No carryover.

requires application of high desorption temperature. In this paper, when desorption was carried out at 300 °C for 5 min, the fatty acids did not show any sign of carryover. Moreover, no cracking on the surface of the fiber was observed. After the fiber was used at least 150 times, the extraction efficiency did not decrease at all. Being chemically bonded to the substrate, the sol–gel-derived TMSPMA-OH-TSO coatings are inherently stable in operations requiring their exposure to high temperature. Thus, the lifetime of the coating is prolonged.

3.3. Matrix effects

The effect of the sample matrix on the extraction of volatile compounds from beer was studied by adding the same amounts of volatile standards to the following matrices: water; 4% ethanol/water (v/v) solution; a concentrated synthetic beer (4% ethanol, pH 4.5); a "volatile-free" beer (4% ethanol, pH 4.5) and a real beer (Beer 1, 4% ethanol, pH 4.5).

Table 2 compares the peak areas of volatile compositions in these matrices. It can be seen from the results in the table that the ethanol concentration has a great negative effect on the extraction. In addition, when the differences in the peak areas between the 4% ethanol/water solution, the concentrated synthetic beer and the "volatile-free" beer standards are studied, it becomes clear that other non-volatile compounds apart from ethanol also play important roles in retaining volatile compositions in the matrix. The influence of beer matrix on the extraction of fatty acids was relatively greater than on the extraction of alcohols and esters. Comparing with the "volatile-free" beer standard, a little decrease in the peak area was also observed for the real beer. These effects can be compensated for by the use of an appropriate internal standard.

Table 3 shows the values of the relative correction factor (F_{is}^{A}) of volatile compositions in these matrices. For SPME analysis, the F_{is}^{A} can be defined as [27]:

$$F_{is}^{A} = \frac{C_{0i}A_s}{C_{0s}A_i} \quad (1)$$

where A_i and A_s are the peak areas of the analyte and internal standard measured by SPME, and C_{0i} and C_{0s} are the initial concentration of the analyte and internal standard spiked in the working standards. According to the results obtained the matrix that did not show significant difference with the real beer should be chosen as standard. It is obvious that the F_{is}^{A}

Table 2
Comparison of peak areas of volatile compositions in various matrices

Volatile compounds	Peak area percentage[a] (%)				
	Water	Water + 4% ethanol	Concentrated synthetic beer	"Volatile-free" beer	Beer 1[b]
Alcohols					
1-Propanol	100	66	79	70	61
Isobutanol	100	68	64	67	64
Isoamyl alcohol	100	66	66	64	63
1-Hexanol	100	53	52	51	51
Linalool	100	76	64	61	57
β-Phenylethanol	100	59	59	59	56
Sum	100	64	63	62	60
Fatty acids					
Acetic acid	100	87	79	77	78
Hexanoic acid	100	68	60	53	52
Octanoic acid	100	63	48	35	34
Decanoic acid	100	80	68	52	50
Sum	100	69	56	43	41
Esters					
Ethyl acetate	100	62	60	59	50
Isobutyl acetate	100	65	63	63	59
Ethyl butyrate	100	71	67	68	62
Isoamyl acetate	100	73	71	66	60
Ethyl hexanoate	100	85	75	74	74
Ethyl lactate	100	83	58	58	55
Ethyl octanoate	100	92	74	77	80
Ethyl decanoate	100	126	119	133	134
Diethyl succinate	100	48	49	47	44
Sum	100	66	61	61	58

[a] Percentage = peak area obtained in other matrix/peak area obtained in the water matrix.
[b] Percentage = (peak area obtained in the spiked beer sample − peak area obtained in the beer sample)/peak area obtained in the water matrix.

Table 3
Relative correction factors (F_{is}^{A}) of volatile compositions in various matrices

Volatile compounds	Matrix								
	Water		Water + 4% ethanol		Concentrated synthetic beer		"Volatile-free" beer		Beer 1 F_{is}^{A}
	F_{is}^{A} (CI)[a]	D	F_{is}^{A} (CI)[a]	D	F_{is}^{A} (CI)[a]	D	F_{is}^{A} (CI)[a]	D	
Alcohols									
1-Propanol	128.3–135.2	Y	109.4–114.0	Y	86.75–88.56	Y	97.13–110.6	N	98.13
Isobutanol	12.36–13.42	Y	9.858–10.94	N	9.434–11.55	N	9.500–10.83	N	10.44
Isoamyl alcohol	3.136–3.403	Y	2.750–3.171	N	2.540–3.076	N	2.750–3.076	N	2.964
1-Hexanol	0.522–0.582	Y	0.538–0.588	N	0.481–0.601	N	0.538–0.626	N	0.587
Linalool	0.062–0.067	Y	0.042–0.051	Y	0.047–0.058	N	0.051–0.059	N	0.056
β-Phenylethanol	1.970–2.165	Y	1.870–1.950	N	1.610–2.000	N	1.527–1.937	Y	1.938
Fatty acids									
Acetic acid	347.1–375.7	Y	199.8–254.7	Y	211.7–268.9	N	224.1–268.9	N	266.9
Hexanoic acid	2.550–2.608	N	2.029–2.250	Y	1.983–2.098	Y	2.221–2.734	N	2.599
Octanoic acid	0.344–0.389	Y	0.319–0.373	Y	0.330–0.419	Y	0.431–0.509	N	0.463
Decanoic acid	0.210–0.218	Y	0.150–0.208	Y	0.172–0.187	Y	0.234–0.280	N	0.267
Esters									
Ethyl acetate	3.980–4.121	Y	3.480–3.837	N	3.443–3.724	N	3.633–3.802	N	3.720
Isobutyl acetate	0.077–0.081	Y	0.063–0.072	N	0.065–0.073	N	0.064–0.075	N	0.072
Ethyl butyrate	1.175–1.208	Y	0.922–0.977	Y	0.983–1.049	Y	0.871–1.010	N	0.913
Isoamyl acetate	0.048–0.051	Y	0.036–0.039	Y	0.036–0.042	N	0.036–0.041	Y	0.042
Ethyl hexanoate	0.070–0.073	Y	0.042–0.052	Y	0.050–0.058	Y	0.057–0.068	N	0.060
Ethyl lactate	169.3–179.4	Y	112.4–122.3	Y	142.1–176.1	Y	133.9–153.4	N	136.1
Ethyl octanoate	0.050–0.054	Y	0.028–0.034	N	0.032–0.040	N	0.033–0.034	N	0.033
Ethyl decanoate	0.286–0.319	Y	0.131–0.146	Y	0.132–0.147	Y	0.095–0.103	N	0.100
Diethyl succinate	1.309–1.419	Y	1.462–1.686	N	1.349–1.570	Y	1.401–1.647	N	1.594

D, significant differences between the F_{is}^{A} of volatile compounds obtained in real beer sample (Beer 1) and that obtained in other matrices. Y, significant difference between them. N, no significant difference between them.
[a] 95% Confidence interval (CI) of mean values of the relative correction factors (F_{is}^{A}).

obtained in the real beer are completely out of the confidence interval of the F_{is}^{A} obtained in the water standard, while most of them are within the confidence interval of the F_{is}^{A} obtained in the "volatile-free" beer standard. There are no evident differences between the F_{is}^{A} obtained in the real beer and that obtained in the "volatile-free" beer standard. Therefore, in the method validation and calibration step, we worked with the "volatile-free" beer described in Section 2.4.

3.4. Method validation

Table 4 summarizes the precisions, limits of detection (LODs) and linear ranges for the analysis of volatile compounds in beer with the sol–gel-derived TMSPMA-OH-TSO fiber. The precision of the method was expressed as the relative standard deviation (RSD). The values obtained were below 7% for all analytes, ranging from 1.68% for isobutanol to 6.18% for decanoic acid, which is considered satisfactory for this type of analysis. The LODs were difficult to determine owing to the effects of the sample matrix. The sensitivity of the SPME-GC system changed with matrix compositions, as shown in Table 2. The determination of LODs in aqueous standard does not give any indication of the LODs in real samples. Therefore, the corresponding LODs were obtained from the "volatile-free" beer standard since little difference in the response was observed relative to the real beer. Owing to the high selectivity and sensitivity of sol–gel-derived TMSPMA-OH-TSO coating, low detection limits were acquired for most of the analytes. A linear regression analysis of the relative peak areas to the internal standard versus the analytes concentration was preformed. The linearity is satisfactory in almost all cases, with the correlation coefficient (r) ranging from 0.9965 to 0.9998. The linear range for each compound was two or three orders of magnitude with the exception of 1-propanol, isobutyl acetate and ethyl butyrate, which have relative low extraction efficiency under the concentration range listed in the table.

3.5. Determination of volatile compounds in beers

The established HS-SPME-GC method was used to determine the content of the volatile compounds in four beer varieties. Fig. 9 shows a typical chromatogram of a real beer sample using the sol–gel-derived TMSPMA-OH-TSO fiber. Volatile compounds were identified from their relative retention times, which were previously determined by injection of standards. The quantitative analysis was carried out by internal standard method using the "volatile-free" beer as standard.

Table 5 shows the mean values of the volatile compounds content in the four beer varieties, the recoveries of the method and the results of the application of the Student–Newman–Keuls test to compare the means for each variety, when significant differences between varieties were obtained from one-way ANOVA test. The recoveries obtained range from 92.8% for diethyl succinate to 105.8% for ethyl hexanoate. The precision for the determination of the real beer samples is also satisfactory for almost all analytes, with the RSD value below 10%. The results of the one-way ANOVA test are positive for all the variables determined, which indicate that there are significant differences among the mean values of the volatile compounds content for the four beer

Table 4
Precisions (RSD), limits of detection (LODs) and linear ranges for the analysis of volatile compounds in beer

Volatile compounds	RSD (%) ($n = 5$)	LODs[a] (μg/l)	Linear range (mg/l)	Regression equation	r
Alcohols					
1-Propanol	3.10	10.4	1.20–64.0	$y = 0.0029 + 0.0009x$	0.9998
Isobutanol	1.68	1.87	0.19–96.0	$y = 0.0157 + 0.0058x$	0.9978
Isoamyl alcohol	2.09	0.28	0.58–58.3	$y = -0.0308 + 0.3833x$	0.9988
1-Hexanol	2.66	0.04	0.02–8.20	$y = 0.0281 + 0.0084x$	0.9966
Linalool	3.66	0.01	0.001–0.70	$y = 0.0084 + 0.0057x$	0.9998
β-Phenylethanol	1.81	0.02	0.12–61.2	$y = 0.0567 + 0.0117x$	0.9965
Fatty acids					
Acetic acid	5.56	35.2	0.63–315	$y = 0.0022 + 0.0006x$	0.9988
Hexanoic acid	3.52	0.27	0.007–3.72	$y = 0.0044 + 0.0008x$	0.9998
Octanoic acid	3.00	0.02	0.11–5.46	$y = 0.0016 + 0.0054x$	0.9992
Decanoic acid	6.18	0.01	0.004–1.80	$y = 0.0092 + 0.0015x$	0.9997
Esters					
Ethyl acetate	1.98	0.37	0.16–81.0	$y = 0.0254 + 0.0122x$	0.9993
Isobutyl acetate	4.87	0.01	0.007–0.34	$y = 0.0030 + 0.0030x$	0.9989
Ethyl butyrate	3.34	0.11	0.02–1.06	$y = 0.0056 + 0.0009x$	0.9986
Isoamyl acetate	5.02	0.01	0.004–1.74	$y = 0.0333 + 0.0254x$	0.9994
Ethyl hexanoate	2.59	0.01	0.001–0.68	$y = 0.0083 + 0.0082x$	0.9991
Ethyl lactate	4.03	16.4	0.56–280	$y = 0.0035 + 0.0012x$	0.9979
Ethyl octanoate	2.52	0.01	0.002–1.02	$y = 0.0280 + 0.0163x$	0.9991
Ethyl decanoate	2.95	0.01	0.002–1.20	$y = 0.0039 + 0.0036x$	0.9998
Diethyl succinate	2.85	0.30	0.17–83.2	$y = 0.0300 + 0.0254x$	0.9995

[a] LODs were estimated on the basis of 3:1 signal-to-noise ratios.

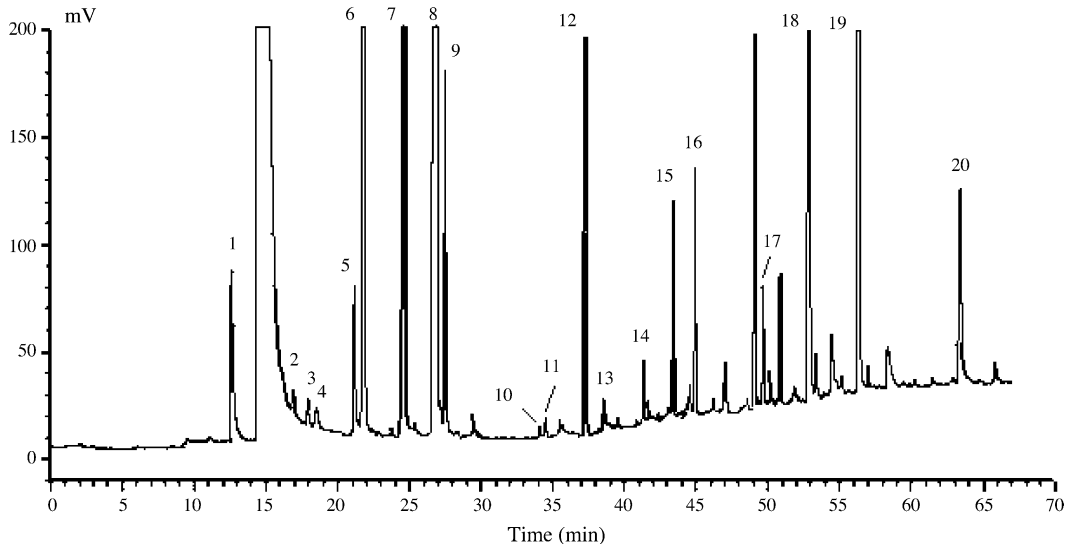

Fig. 9. HS-SPME-GC analysis of a real beer sample using the sol–gel-derived TMSPMA-OH-TSO fiber. SPME-GC conditions are the same as in Fig. 4. Peaks: (l) ethyl acetate; (2) isobutyl acetate; (3) ethyl butyrate; (4) 1-propanol; (5) isobutanol; (6) isoamyl acetate; (7) 4-methyl-2-pentanol; (8) isoamyl alcohol; (9) ethyl hexanoate; (10) ethyl lactate; (11) 1-hexanol; (12) ethyl octanoate; (13) acetic acid; (14) linalool; (15) ethyl decanoate; (16) diethyl succinate; (17) hexanoic acid; (18) β-phenylethanol; (19) octanoic acid; (20) decanoic acid.

Table 5
Mean values of the volatile compounds content, the recoveries of the method and results of Student–Newman–keuls test for means comparisons

Volatile compounds	Beer 1		Beer 2		Beer 3		Beer 4		Recovery b (%)
	Mean ± CI a (mg/l)	RSD (n = 5)	Mean ± CI a (mg/l)	RSD (n = 5)	Mean ± CI a (mg/l)	RSD (n = 5)	Mean ± CI a (mg/l)	RSD (n = 5)	
Alcohols									
1-Propanol	9.28 ± 0.52 c	6.38	3.84 ± 0.16	4.69	7.39 ± 0.50	7.80	10.01 ± 0.63 c	7.19	103.5
Isobutanol	12.61 ± 0.72 c	6.51	3.72 ± 0.16	4.83	11.43 ± 0.64 c	6.40	11.42 ± 0.64 c	6.41	102.6
Isoamyl alcohol	65.07 ± 2.70 c	4.73	47.34 ± 0.84	2.02	75.49 ± 1.23	1.87	68.99 ± 2.01 c	3.33	100.1
1-Hexanol	0.02 ± 0.00	9.04	0.10 ± 0.00	6.29	0.08 ± 0.00	3.33	0.12 ± 0.00	2.98	93.9
Linalool	0.01 ± 0.00 c	4.37	0.01 ± 0.00 c	7.73	0.03 ± 0.00	4.63	0.02 ± 0.00	6.16	100.9
β-Phenylethanol	17.23 ± 0.93 c	6.17	10.46 ± 0.56	6.13	26.92 ± 1.46	6.20	18.81 ± 0.95 c	5.74	102.3
Sum	104.2		65.47		121.3		109.4		
Fatty acids									
Acetic acid	48.55 ± 3.27	7.68	35.98 ± 2.03	6.42	77.22 ± 2.47 c	3.65	84.95 ± 5.50 c	7.38	102.4
Hexanoic acid	2.79 ± 0.13 c	5.45	3.83 ± 0.15	4.56	2.29 ± 0.14	6.96	2.69 ± 0.16 c	6.84	104.4
Octanoic acid	3.98 ± 0.27 c	7.61	4.07 ± 0.28 c	7.94	2.77 ± 0.19	7.64	1.81 ± 0.13	8.34	103.1
Decanoic acid	1.63 ± 0.13 c	9.45	1.69 ± 0.14 c	9.27	0.49 ± 0.03	7.95	0.38 ± 0.02	8.59	105.3
Sum	56.95		45.57		82.77		89.83		
Esters									
Ethyl acetate	9.42 ± 0.35 c	4.21	6.34 ± 0.51	8.07	13.24 ± 0.64	5.50	8.76 ± 0.85 c	9.68	102.8
Isobutyl acetate	0.01 ± 0.00 c	0.33	0.03 ± 0.00	7.64	0.01 ± 0.00 c	7.34	0.02 ± 0.00	5.95	97.5
Ethyl butyrate	0.09 ± 0.00 c	5.11	0.15 ± 0.01 d	7.38	0.15 ± 0.01 d	7.85	0.10 ± 0.01 c	5.12	99.6
Isoamyl acetate	0.44 ± 0.02 c	4.69	0.31 ± 0.02	7.85	0.48 ± 0.03 c	6.97	0.26 ± 0.02	6.71	105.4
Ethyl hexanoate	0.14 ± 0.01 c	4.79	0.26 ± 0.01	6.46	0.15 ± 0.00 c	3.79	0.12 ± 0.00	4.81	105.8
Ethyl lactate	8.51 ± 0.29 c	3.86	5.04 ± 0.10	2.23	5.84 ± 0.07	1.45	8.26 ± 0.15 c	2.02	100.1
Ethyl octanoate	0.22 ± 0.01	5.40	0.25 ± 0.01 c	2.96	0.26 ± 0.01 c	4.07	0.18 ± 0.01	6.60	96.2
Ethyl decanoate	0.40 ± 0.01 c	3.22	0.42 ± 0.03 c	8.71	0.18 ± 0.01	7.36	0.08 ± 0.00	7.41	97.9
Diethyl succinate	0.92 ± 0.04 c	5.52	0.52 ± 0.04	7.76	1.85 ± 0.11	6.63	1.02 ± 0.08 c	8.50	92.8
Sum	20.15		13.32		22.16		18.80		

a: 95% Confidence interval (CI) of the mean values of the volatile compounds content. b: Recovery = (amount founded in the spiked sample − amount founded in the sample)100/amount added. c and d: Mean values in the same row indicate that there are no significant differences between them ($p < 0.05$).

varieties. Although Beer 1 and Beer 4 are very close, the mean values of 1-hexanol, linalool, acetic acid, octanoic acid, decanoic acid, isobutyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate content are evidently different in the beers of the two varieties.

Alcohols are quantitatively the largest group of the volatile compounds in the four beer varieties. They can be recognized by their strong and pungent smell and taste. It can be seen that the alcohols predominated in the four types of beers are isoamyl alcohol and β-phenylethanol. The content of these two alcohols are especially high in Beer 3. The total content of alcohols in the four beer varieties is evidently different from each other, although that between Beer 1 and Beer 4 are very close. In addition, the total content of alcohols in Beer 2 is especially low compared to the other three varieties.

The fatty acids constitute an important group of aroma compounds that can contribute with fruity, cheesy, and fatty odors to the beer's sensory properties. They also contribute to bitterness, astringency and rancidity. Beer 3 and Beer 4 have higher content of acetic acid, while fewer amounts of octanoic acid and decanoic acid than Beer 1 and Beer 2. Also the concentration of hexanoic acid in Beer 2 is highest among the four varieties.

Esters are formed primarily during the fermentation and are a characteristic of young beers. Although the content of most of esters is relatively low, the contribution of this group to the whole flavour/aroma is great. They are often characterized by their fruity flavour. The esters predominated in the four beer varieties are ethyl acetate, isoamyl acetate, ethyl lactate and diethyl succinate. The total content of esters in Beer 2 is much lower than the other varieties. Besides, Beer 3 possessed the maximum of ethyl acetate, isoamyl acetate and diethyl succinate in the four varieties, while Beer 4 held the least of isoamyl acetate, ethyl hexanoate, ethyl octanoate and ethyl decanoate.

3.6. Identification of the major aroma contributing substances

Although the volatile compounds content in these beer varieties are determined, it is not enough to evaluate the actual contribution of each analyte to the overall beer flavour only by this information. The relationship of volatile compounds and beer aroma can be established by means of aroma description of each compound. The contribution of each volatile compound to the overall beer aroma can be quantified via its aroma index (*I*), which was calculated by dividing compound

Table 6
Odour description, odour threshold, and the aroma index (*I*) of the aroma compounds of the four beer varieties

Volatile compounds	Odour description	Odour threshold (mg/1)	Aroma index (*I*)			
			Beer 1	Beer 2	Beer 3	Beer 4
Alcohols						
1-Propanol	Alcohol, ripe fruit[a]	800[b]	0.01	0.005	0.01	0.01
Isobutanol	Alcohol, winelike, nail polish[a]	200[b]	0.06	0.02	0.06	0.06
Isoamyl alcohol	Fusel oil[c]	70[b]	0.92	0.68	1.10	0.99
1-Hexanol	Herbaceous[d]	4[b]	0.004	0.02	0.02	0.03
Linalool	Flowery, muscat[e]	0.08[b]	0.13	0.09	0.34	0.23
β-Phenylethanol	Lily[c]	125[b]	0.14	0.08	0.22	0.15
Fatty acids						
Acetic acid	Vinegar[d]	200[f]	0.24	0.18	0.39	0.42
Hexanoic acid	Rancid, grass, fruity[d]	8[f]	0.35	0.48	0.29	0.34
Octanoic acid	Fatty acid, dry, dairy[d]	15[f]	0.26	0.27	0.18	0.12
Decanoic acid	Fatty acid, dry, woody[d]	10[f]	0.16	0.17	0.05	0.04
Esters						
Ethyl acetate	Sweet, fruity[a]	30[b]	0.31	0.20	0.44	0.29
Isobutyl acetate	Flowery[e]	1.6[b]	0.006	0.02	0.01	0.01
Ethyl butyrate	Apple/jonquil[c]	0.4[b]	0.23	0.38	0.37	0.25
Isoamyl acetate	Fruit/sweet[c]	1.2[b]	0.37	0.26	0.40	0.21
Ethyl hexanoate	Banana, green apple[a]	0.21[b]	0.68	1.20	0.72	0.57
Ethyl lactate	Buttery, butterscotch, fruit[a]	250[b]	0.03	0.02	0.02	0.03
Ethyl octanoate	Pipe fruits, pear, sweety[d]	0.9[b]	0.25	0.28	0.28	0.20
Ethyl decanoate	Sweety, fruity, dry fruits[d]	1.5[b]	0.26	0.28	0.12	0.05
Diethyl succinate	Cheese, earthy, spicy[d]	1.2[g]	0.77	0.43	1.50	0.85

Odour description and odour threshold reported in the literature [28,30–35]. Superscript corresponds to numbered reference.

[a] [32].
[b] [33].
[c] [30].
[d] [28].
[e] [31].
[f] [35].
[g] [34].

concentration by its corresponding odor threshold in beer, in an aqueous alcohol solution or in water, depending on the information available in the literature [28]. Generally, compounds that present in concentrations higher than their odor threshold, namely that exhibit $I > 1$ were considered to contribute individually to the beer aroma and were designated as would-be impact odorants. Furthermore, in terms of Meilgaard's suggestion of the sensorial contribution to the overall aroma of a substance, when its concentration is at least 20% of the flavor threshold ($I > 0.2$), it should be considered [29]. Table 6 shows the odor description, odor threshold, and the aroma index (I) of the aroma compounds of the four beer varieties.

Higher alcohols are reported to contribute more to the intensity of the odor of the beer than to its quality. For the six alcohols studied, only isoamyl alcohol exhibits an aroma index close to 1. This compound is correlated with the fusel oil odor. In addition, the presence of linalool ($I > 0.2$) in Beer 3 and Beer 4, which belongs to the monoterpene alcohol, contributes to the flowery and muscat odors. The presence of β-phenylethanol ($I > 0.2$) in Beer 3 can give the beer a lily flavor. Aroma indexes of the other alcohols are far below 1 due to their very high flavor threshold. These compounds contribute weakly to the overall odor of beers on the basis of their low aroma indexes.

Aroma indexes of the four fatty acids studied are all below 1 due to the high flavor threshold, indicating that these compounds can not contribute individually to beer aroma, but the presence of them in beer can also exhibit their importance as flavor compounds.

Esters contribute favorably to beer aroma as fruity notes, and may contribute to the fruity characteristics of beer. Particularly, acetate esters are correlated with the freshness and fruitiness of young beers. In Beer 2, only ethyl hexanoate exhibits an aroma index higher than 1 ($I = 1.2$), which is associated with fruit and anise notes. In Beer 3, only diethyl succinate possesses an aroma index higher than 1 ($I = 1.5$). This compound contributes to the fruity and floral flavour. In addition, the presence of the other five esters, ethyl acetate, ethyl butyrate, isoamyl acetate, ethyl octanoate, and ethyl decanoate, although exhibiting aroma indexes just above 0.2, contribute to the fruity character of beers too.

The proposed methodology, which does not include the use a sensory panel and GC-olfactometric studies, seems to be adequate, as a preliminary step, to establish the major aroma contributing substances of beer. However, further steps, such as GC-olfactometric studies are necessary to confirm the impact of the odour-active compounds already identified.

4. Conclusion

A validated and accurate HS-SPME/GC method using sol–gel coated TMSPMA-OH-TSO fiber was presented for the analysis of both major and minor volatile compounds in beer. The novel coating was proved to be suitable for simultaneous extraction of both polar alcohols and fatty acids and nonpolar esters. It exhibits better sensitivity to most of the investigated analytes compared to commercial PDMS, PDMS-DVB and PA fibers. High thermal stability and long lifetime are also characteristics of the new fiber. The established internal standard method using a "volatile-free" beer as standard avoided the influence of the implicated sample matrix on the extraction, and hence improved the accuracy of the analytical procedure. The recoveries obtained range from 92.8 to 105.8%, with a mean value of 100.9%. The method proposed also showed satisfactory linearity, precision and detection limits.

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