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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.