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REVIEW ARTICLE

Application of solid phase extraction techniques to analyse volatile compounds in wines and other enological products

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Abstract Aroma compounds are most closely associated with the volatile fraction of foods. Common analytical separation procedures employed to analyse volatile compounds need, even today, that prior to GC analysis of an aroma or a fragrance, these compounds be concentrated or/and isolated from the non-volatile matrix. This step constitutes a problem that has still not been satisfactorily resolved and for this, several sample preparation methods can be found into the bibliography. This review gives a brief overview of solid phase extraction techniques to analyse volatiles. Procedures such as solid phase extraction (SPE), solid phase microextraction (SPME), stir bar sorptive extraction (SBSE), and the recent solid phase dynamic extraction (SPDE) will be discussed and critically evaluated. Contemporary applications of these techniques to the study of volatile compounds in wine and other enological products will be presented.

Keywords Volatile compounds · Enological products · SPE · SPME · SBSE · SPDE

Introduction

The study of the volatile fraction in enological products has become necessary and is more than sufficiently justified, considering these compounds make a major contribution to the consumer's overall perception of the quality of particular food and drink products. In fact, these complex volatile compounds largely determine the acceptance or rejection of many products by the consumer. In addition, product characteristics known as "off-flavours", caused by the presence of volatiles that give rise to disagreeable odours and flavours, often imply microbial contamination; therefore the study of volatiles becomes part of the larger subject of food safety.

It must also be borne in mind that the volatile fraction of these food and drink products tends to be conditioned by all the different circumstances of their production, and as a result the characterisation and differentiation of these products may be possible on the basis of the volatile fraction. There is ample evidence in numerous studies that it is possible to establish clear relationships between the following aspects: the raw material employed, the place where that material originated, the process of production followed, etc. and the volatile fraction of the product [1–6].

The determination of the volatile fraction is normally performed by gas chromatography (GC), a technique which in recent years has made great advances. Nevertheless, it should be recognized that, despite this, in the majority of the cases, determination by GC needs to be preceded by a prior stage of sample preparation. The need for sample preparation is imposed by various factors, most notably the following:

- The low concentrations in which the analytes of interest are present.
- The great chemical variety that these present: acids, alcohols, esters, ketones and many other types of compound that could be found.
- The fact that in the majority of cases these compounds are found in matrices of great complexity, as is the case of enological products.
- And last, the low chemical stability of the compounds, together with the limited specificity of the systems of detection.

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All of these considerations lead us to seek techniques that allow their fractionation and concentration prior to the analysis by CG, making sample preparation a critical step when characterising the volatile fraction of these products.

If we first consider how to classify the various different techniques used for sample preparation prior to GC analysis, we find a number of different classifications in use. One of the criteria adopted when dealing with this classification is the physico-chemical property on which the isolation of these analytes is based [7]. Thus we find that there are techniques based on the following:

- The volatility of the analytes: distillation processes and head space techniques.
- Solubility of the analyte in certain organic solvents: soxhlet extraction, liquid-liquid extraction (LLE), supercritical fluid extraction (SFE), solid phase extraction (SPE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE).
- The adsorption and absorption of the analyte on a particular material: solid phase extraction (SPE), solid phase microextraction (SPME), stir-bar sorptive extraction (SBSE) and solid phase dynamic extraction (SPDE).
- And the last group where we find those techniques that make use of both properties: the most notable of these is simultaneous extraction-distillation, together with other instrumental configurations where several of the previously mentioned techniques are combined.

In this review we shall consider the application to enological analysis of some of the techniques included in the second group that share the common feature of being based on the employment of adsorptive and/or sorptive materials in order to trap the analytes of interest. SPE is still employed to analyse volatile compounds, although it has now been largely replaced by methodologies that are less aggressive and more sensitive, such as SPME, SBSE and the contemporary SPDE.

SPE

SPE can be directly applied to isolate and concentrate volatile compounds from liquid samples. This technique, introduced in the eighties, is based on the selective retention of some analytes and their subsequent elution by an appropriate solvent.

Depending on the type of sorbent and on the characteristics of the analyte, a series of physical and chemical interactions are established that allow the analyte of interest to be separated from the rest of the components of the sample. The selectivity of the separation will be conditioned by the type of sorbent and eluent employed.

The sorbents utilized are similar to the stationary phases employed in liquid chromatography, and can be grouped into polar, non-polar and ion exchange types. The choice of the sorbent depends on the nature of the analyte, of the matrix, and of the possible interferents. Of particular importance are the silica and polymeric sorbents, such as styrene-divinylbenzene copolymers. The silica sorbents are characterized by presenting a low loading capacity and a high consumption of solvents and time; in addition, in some cases, we can be faced with irreversible sorption and with the degradation of certain analytes [8]. The second type, based on styrene-divinylbenzene polymers, are characterized by presenting a greater loading capacity, more stability against extreme pH values, plus the capacity for employment in both reversed and normal mode [8].

SPE is a technique that has great applicability to enology, having in some cases displaced liquid-liquid extraction.

Application of SPE to enological products

Wada and Shibamoto [9] studied the extraction of odorant from red wines using Porapak Q columns (ethylvinylbenzene-divinylbenzene copolymer). Different solvents were tested, and dichloromethane was found to be the best, with recoveries near to 100%.

Different authors have employed styrene-divinylbenzene copolymers in order to study the volatile fraction of some enological products [8, 10–12]. López and others [10] used LiChrolut-EN (styrene-divinylbenzene copolymer) for the extraction of 27 volatiles from wine. Wine measuring 50 mL was extracted using 200 mg of resin. The authors concluded that the SPE analytical methodology was satisfactory for the study of wine aroma.

Later, Culleré and others [8] using the same type of sorbent carried out the fractionation of wine flavour. First, volatile compounds were isolated and concentrated with dichloromethane and then they were fractioned using a second LiChrolut-EN cartridge. A first fraction, rich in ethyl esters and some other non-polar compounds, was eluted from this second cartridge with pentane. The second fraction, which concentrates the alcohols and some volatile phenols, was eluted with pentane dichloromethane (9:1), while the third fraction, eluted with dichloromethane, is rich in fatty acids, vanillin derivates and lactones.

Genovese et al. [12] used three extraction methods for the determination of the aroma composition of red wines: separation of the alcoholic fraction from the aqueous phase by salting out and liquid–liquid extraction with trichlorotrifluoroethane; liquid–liquid extraction with dichloromethane; and solid-phase extraction using 800 mg of LiChrolut EN resin with pentane-dichloromethane (20:1) and dichloromethane. These authors concluded that, owing to the variety of the chemical species that constitute the aroma of red wines, only the combination of different extraction techniques can allow their complete evaluation.

Several studies can be found into the bibliography about silica sorbents and volatile compounds of enological products [13–15]. Lukic and others investigated the adsorption properties of octadecylsilica sorbent for the determination of some varietal and fermentation aroma compounds in grape distillates [13]. In this case, a 3-mL volume of sample was diluted to 25 mL and dichloromethane was selected as eluent. The SPE method was validated and the possible matrix effect of acetic acid and ethanol content was evaluated. In general, good recoveries were obtained, except for some alcohols such as 2-phenylethanol, *cis*-2-hexen-1-ol and *cis*-3-hexen-1-ol. These authors concluded that the influence of the non-polar alkyl chain in the straight-chain alcohol or acid molecule prevails over the hydrophilic hydroxyl or carboxylic functional group and is responsible for high SPE recoveries. This is in agreement with Ferreira and others [14], who found that silica-based sorbents were suitable for the extraction of analytes that show a Bronsted–Lowry acid character.

Among the volatile compounds that are responsible for the floral character of wines, some monoterpenes can be found. Their concentrations in grapes and wines are influenced by several factors such as grape variety, climate, viticultural and enological practices, etc. Obtaining a 'terpenic profile' is extremely useful for differentiating the genuinely monovarietal wines from those made by a mixture of several other varieties. Therefore, the determination of the above-mentioned profiles is a valuable tool for detection of fraud.

A first study using a C18 cartridge to determine the content of terpenes (free and glycosidically linked) in wines from the white cultivar Muscat Lefko from the Greek island of Samos was carried out by Karagiannis and others [16]. Extraction of free and mono and dihydroxylated terpenes was performed with dichloromethane, whereas extraction of trihydroxylated and glycosidically linked terpenes was performed with methanol.

Later, Piñeiro and others [17] compared different C-18 cartridges to others with a styrene-divinylbenzene solid phase for the determination of monoterpenic derivates in wines. The best results were obtained for two styrene-divinylbenzene cartridges, LiChrolut EN and Strata SDB-L, using dichloromethane as eluent.

Lactones are a group of compounds that make an important contribution to the aroma in wines [18]. Ferreira and others have developed a SPE method for the selective extraction of aliphatic lactones in wines [19]. For this, 200 mg Bond Elut-ENV resins are conditioned by rinsing with 2 ml of methanol and 4 ml of water. After this, 50 ml of wine is passed through the cartridge. The interferences are removed with a mixture of methanol-water 40:60 (v/v) enriched with 1% (w/v) $NaHCO_3$. Lactones are eluted with 1.8 ml of dichloromethane. Later, this volume is concentrated to 0.15 ml in a bath at 47 °C. Following this methodology, it was proved possible to quantify eight C8–C12 aliphatic lactones in wines, with recoveries higher than 75% and excellent precision (average RSD 3.5%) and linearity ($r^2 \geq 0.996$).

Recently, Campo and others [20] have employed a SPE methodology to the determination of four powerful aroma compounds (2-, 3- and 4-methylpentanoate and ethyl cyclohexanoate) in wine, whisky and brandy. 100 mL of sample is extracted using a LiChrolut EN bed. A water–methanol solution is employed to eliminate the major compounds and dichloromethane is used as elution solvent.

On the other hand, cork taint is one of the most significant organoleptic defects in wines. Cork is the traditional material used to produce stoppers for wine bottling. Cork stoppers are obtained from the bark of cork oak (Quercus suber), which grows mainly in Mediterranean countries. Cork is a natural product and is not inert, so it can interact with wine, sometimes modifying its flavour and can even cause organoleptic defects. This is the case of the musty/mouldy taint, traditionally known as cork taint. Some of the compounds involved in this defect may originate in the cork stopper. The mainly responsible compound is 2,4,6-trichloroanisole (TCA), but also, to a lesser extent 2,3,4,6-tetrachloroanisole (TeCA) and 2,3,4,5,6-pentachloroanisole (PCA) are also responsible. Chloroanisoles usually arise from *O*-methylation of chlorophenols, as a detoxification method, by various microorganisms, especially fungi, under particular conditions of temperature and humidity. Chlorophenols are often present because of the packaging, the fungicides, herbicides or wood preservatives that are used in wineries, or the practices of some cork stopper manufacturers, such as using hypochlorite as a cork bleaching agent. The wines could also become contaminated in the cellar. For instance, chlorophenol contamination is possible in the pallet crates used for bottle storage. These chlorophenols may develop into chloroanisoles, which could contaminate the cellar atmosphere and, consequently, contaminate the wine during the winery operations. The presence of chloroanisoles in wine is due to their ability to migrate from cork to wine if they are present in contaminated cork. Taking into account the bad odour (off-flavours) and low sensorial perception threshold of these compounds, between 1 ng/L and 50 ng/L, this migration will imply serious consequences for the organoleptic properties of affected wine, undermining its quality.

The determination of chloroanisoles in wines, therefore, has stimulated extensive research over the last decade to develop methods as sensitive as the human sensory threshold. To avoid the financial losses caused by this musty off-flavour, it is very important to prevent this defect by the effective control of chloroanisoles in cork. This control requires appropriate analytical methods which must pro-

vide enough sensitivity and selectivity as well as good repeatability and recovery. Gas chromatography is the most common technique used in these studies, usually coupled to either a mass spectrometer detector, or to an electron capture detector (ECD) and, recently, coupled to an atomic emission detector. However, due to low chloroanisole contents in cork, a prior extraction and concentration step is necessary, and several different techniques have been employed to this end. Insa and others developed a solid-phase extraction method for the determination of 2,4,6-trichloroanisole (TCA) and 2,4,6-tribromoanisole (TBA), which seem to be the compounds that contribute most to this problem [21]. In this method, 50 mL of wine was extracted with 50 mg of LiChrolut EN resins, using 0.6 ml of dichloromethane as eluent. A 40-μL aliquot of this extract was injected into the GC system using a programmed temperature vaporizing injector (PTV) as large volume injection technique. The SPE method was validated, obtaining RSD values lower than 6% and recoveries higher than 80%. The detection limits were 0.2 and 0.4 ng/L for TCA and TBA, respectively. These values are below the odour detection threshold of these compounds.

Another volatile compound reported in several products such as wines, and which is characterized by a fungus odour, is 1-octen-3-one. Its determination in wines has been recently optimized by Culleré and others [22]. The analyte is selectively preconcentrated in a 90-mg LiChrolut-EN solid phase extraction cartridge and, after this, is derivatized with O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) in the same cartridge. The elution of the oximes of the analyte is carried out with 2 mL of pentane. After the optimization of the SPE method, the authors evaluated linearity, limits of detection and accuracy. Good linearity was obtained up to 900 ng/L, with a squared correlation coefficient of 0.9990. The precision obtained was satisfactory and the method is free from matrix effect.

Among volatile compounds that negatively affect the aroma of wine are volatile phenols such as ethylphenols (4-ethylphenol and 4-ethylguaiacol) and vinylphenols (4-vinylphenol and 4-vinylguaiacol). These compounds are considered part of the aroma composition of wines and can produce unpleasant odours, affecting negatively the quality of the wine. Dominguez and others [23] developed a SPE method to determine volatile phenols in fino sherry wine, a typical white wine from the Jerez–Xérès–Sherry Denomination of origin region [24]. Briefly, the cartridges employed were Lichrolut EN (200 mg), conditioned with 5 ml of methanol and 3 ml of water. A total of 10 ml of wine was used. The cartridges were rinsed with 0.6 ml of water and dried with helium. Finally, 2.5 ml of dichloromethane was applied as eluent. The SPE methodology optimized by these authors was compared to two other methods based on liquid-liquid extraction. The SPE method showed a higher selectivity and sensitivity for these compounds than the others. Further, practical advantages in respect of sample volume, time, and number of samples extracted in a single extraction session, lead the authors to select the SPE methodology for the determination of volatile phenols in fino sherry wine.

Vinegar is used not only as a condiment but also as ingredient in many food products, particulary sauces and dressings. Due to the diversity of vinegars available in the market and the increase in demand, it has been considered necessary to investigate reliable analytical methods to establish criteria for determining quality and origin. In vinegars, Charles and others [25], after neutralization with NaOH, studied the representativeness of four extracts obtained by liquid-liquid extraction with dichloromethane and SPE using XAD-2, a mixture of XAD-2 and XAD-7, and Extrelut resins. Panelists indicated that the liquid-liquid extract was the most representative of the volatile fraction of vinegar.

Later, Morales and others compared two SPE methods to a liquid–liquid extraction method with dichloromethane for the analysis of volatile compounds in vinegars [26]. For the SPE, two cartridges were tested: a LiChrolut EN and a Bond Elut ENV. Dichloromethane was used as eluent in both cases. Liquid-liquid extraction showed a higher extraction than the SPE methods only for furfuryl alcohol. In the case of SPE methods, Bond Elut ENV cartridges gave low responses for furanic compounds, so the authors selected LiChrolut EN cartridges for the extraction of volatile compounds of vinegars.

Table 1 includes various different applications of SPE to wine and other enological products.

SPME

Solid phase microextraction (SPME) is an extraction technique developed by Pawliszyn [27–29] at the beginning of the 1990's.

It is based on the establishment of a partition equilibrium of the analytes between a polymeric stationary phase, which covers a fused silica fibre, and the matrix of the sample, Fig. 1. This technique does not require the employment of organic solvents, thus eliminating all the disadvantages that this entails. It is a simple, rapid and inexpensive technique in which the extraction and concentration processes are performed simultaneously, Fig. 2. Further, only small volumes of sample are required. The device can be coupled easily to a gas chromatography system and, with some modifications, to a high performance liquid chromatography (HPLC) system.

The SPME device consists of a small fibre of fused silica (usually of 1 cm length and 0.11 mm internal diameter),

Table 1 SPE analytical conditions used to analyse volatile compounds in enological products

Authors	Matrix	Column	Analytes	Extraction conditions
Wada and Shibamoto [9]	Red wine	Ethylvinylbenzene-divinylbenzene copolymer	Flavour	*Solvent:* dichloromethane
López et al. [10]	Wine	Styrene-divinylbenzene copolymer	Flavour	*Sample volume:* 50 mL; *Solvent:* dichloromethane
Culleré and others [8]	Wine	Styrene-divinylbenzene copolymer	Flavour	*Sample volume:* 75 mL; *Solvents:* dichloromethane, pentane and pentane:dichloromethane (9:1)
Lukic et al. [13]	Grape distillate	Octadecylsilica	Flavour	*Sample volume:* 3 mL diluted to 25 mL; *Solvent:* dichloromethane
Dieguez et al. [15]	Spirits	Silica	Volatile organic acids	*Sample volume:* 50 mL; *Solvent:* dichloromethane
Genovese et al. [12]	Red wine	Ethylvinylbenzene-divinylbenzene copolymer	Flavour	*Sample volume:* 50 mL; *Solvents:* pentane:dichloromethane (20:1) and dichloromethane
Karagiannis et al. [16]	White wine	Silica	Terpenes	*Sample volume:* 25 mL; *Solvents:* dichloromethane and methanol
Piñeiro et al. [17]	White wine	Styrene-divinylbenzene copolymer	Terpenes	*Solvent:* dichloromethane
Ferreira et al. [19]	Wine	Styrene-divinylbenzene copolymer	Aliphatic lactones	*Sample volume:* 50 mL; *Solvent:* dichloromethane
Campo et al. [20]	Wine, whisky and brandy	Styrene-divinylbenzene copolymer	Flavour	*Sample volume:* 100 mL; *Solvent:* dichloromethane
Insa et al. [21]	Wine	Styrene-divinylbenzene copolymer	Anisoles	*Sample volume:* 50 mL; *Solvent:* dichloromethane
Dominguez et al. [23]	White wine	Styrene-divinylbenzene copolymer	Volatile phenols	*Sample volume:* 10 mL; *Solvent:* dichloromethane
Charles et al. [25]	Vinegar	Styrene-divinylbenzene copolymer	Flavour	–
Morales et al. [26]	Vinegar	Styrene-divinylbenzene copolymer	Flavour	*Solvent:* dichloromethane

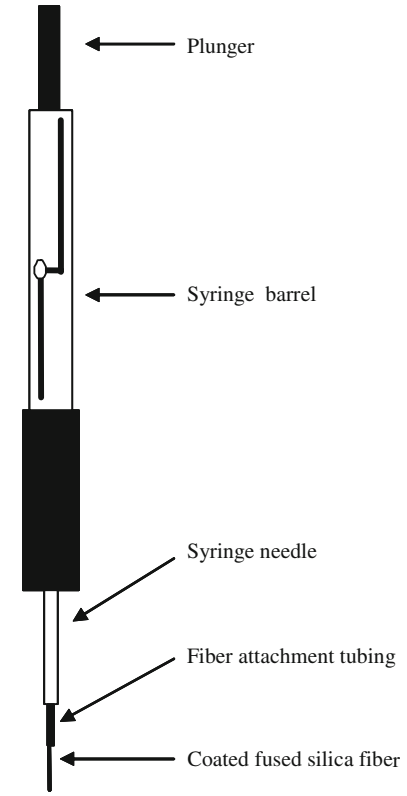

Fig. 1 Diagram of an SPME device

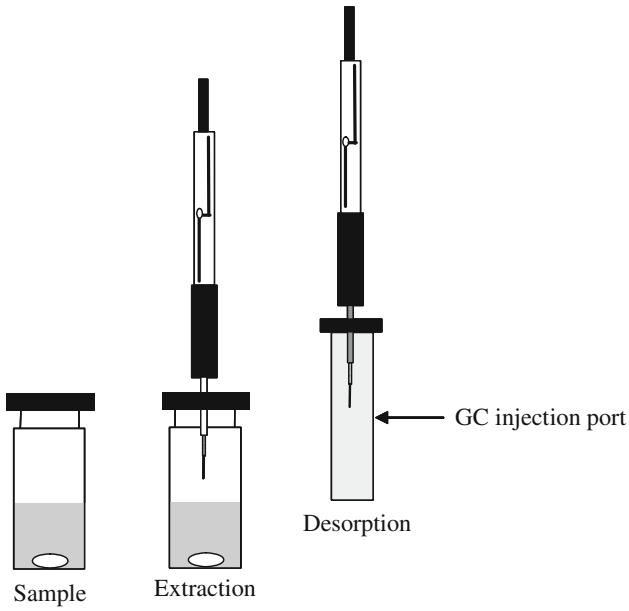

Fig. 2 Stages of the solid phase microextraction process

normally coated with a polymeric phase. For protection, this fibre is mounted in a type of modified syringe or holder, Fig. 1. The analytes are retained by the fibre until the system reaches equilibrium.

Solid phase microextraction comprises two stages: extraction and desorption [7]. In the first stage, the sample

is placed in a vial. The vial is sealed with a septum and a capsule. The needle of the syringe with the fibre inside it perforates the septum. Then, by actuating the plunger, the fibre is brought into contact with the aqueous sample or with the headspace that exists over the liquid. After a predetermined time, the fibre is withdrawn and inserted back into the needle and the syringe is withdrawn from the sampling vial. In the stage of desorption, immediately following, the syringe is inserted in the injector of an analytical instrument (GC or HPLC), where the analytes are desorbed thermally or by solution in the mobile phase, according to the instrumental technique employed. This desorption stage takes 1–2 min to complete. In HPLC the standard injector must be replaced by a special device.

There are three basic types of solid phase microextraction: direct extraction, headspace extraction, and extraction utilising a protective membrane. The thermodynamics predict the effects produced by certain extraction conditions on the distribution of the analytes between the fibre and the matrix. These parameters are as follows: the polymeric coating of the fibre, extraction temperature and time, saline effect, pH of the sample, volume of the sample, volume of the head space, agitation of the sample, and shape of the vial. The chemical nature of the analyte determines what type of phase must be utilised in the extraction. Currently, various types of stationary phase are commercially available, with different thicknesses and polarities that show affinity for different analytes. In Fig. 3 the characteristics of some of the more frequently utilized phases are indicated [29]. Polydimethylsiloxane (PDMS) is a non-polar phase that presents great affinity for apolar compounds, although it can be utilized to extract moderately polar compounds. Polyacrylate (PA) is a phase that is suitable for more polar compounds. In addition to these two general types of phase, other coatings of more specific materials, and mixed phases that have properties complementary to those of the PDMS and PA phases, have been developed. In this group are included the polydimethylsiloxane/polydivinylbenzene (PDMS/DVB) phases; polyethyleneglycol/polydivinylbenzene (Carbowax/DVB), and Carboxen/PDMS. These fibres, more polar than those of PA, are suitable for extracting more polar compounds like alcohols and ethers. In addition, fibres of Carboxen/PDMS have a larger surface area and are suitable for the extraction of volatile organic compounds (VOCs).

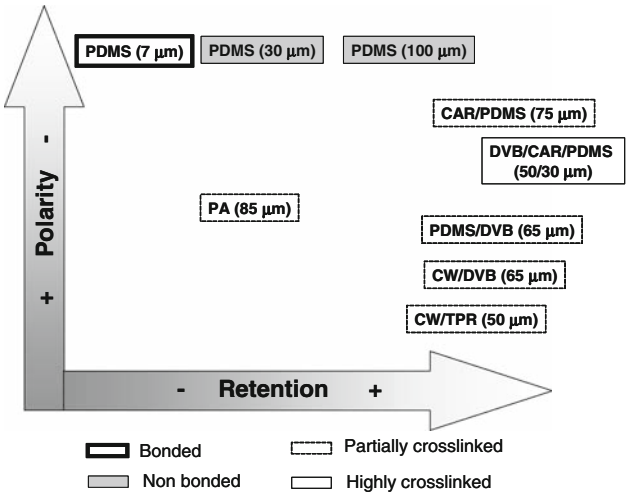

Fig. 3 Properties of the various stationary phases utilized in SPME (Reprinted from [29] with permission from Elsevier)

Solid phase microextraction (SPME) is now being utilized satisfactorily, in combination with gas chromatography (GC) and GC-mass spectrometry (GC-MS), and is being applied to a great variety of compounds in the analysis of wines.

Application of SPME to enological products

In 1997, De la Calle and others [30] studied several different fibres in the application of SPME for the extraction of the components of wine bouquet. Polyacrylate fibres (PA-85) achieved the most complete bouquet profiles. About 90 substances were identified by capillary gas chromatography-MS and their retention factors were calculated to allow peak identification in capillary-GC-FID chromatograms. After 1 year, headspace solid-phase microextraction (HS-SPME) was also studied and optimized for the GC analysis of wine aroma compounds [31] and the results were compared with those obtained using the direct sampling mode (DI-SPME) and using liquid–liquid extraction. The aromatic patterns obtained by HS-SPME-GC were applied to the chemometric classification of wine varieties. The results obtained using the three techniques were similar. However, HS-SPME presented additional advantages: a greater sensitivity in the determination of terpenoids, and the lifetime of the SPME fibre is more than three times longer than in direct sampling mode because the fibre is not in contact with the sampling matrix and thus is not contaminated by strongly polar compounds, ethanol and salts.

Tat et al. [32] studied the performance of different fibres developed in recent years for solid-phase microextraction. The fibres were evaluated for their sensitivity and repeatability; the results showed a notably different behaviour for the different solid-phases, both for the different zones of the chromatogram and for different levels of concentration. A divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre coating appeared the most suitable for the analysis of the aromatic fraction of wines in its totality. For specific applications, the choice of a suitable solid-phase depends on the class of compounds be analysed.

Whiton and Zoecklein [33] studied how the quantitation can be affected by sample matrix and sampling conditions.

Tests with model solutions containing a range of typical wine volatiles demonstrated that increasing temperature and sampling time can increase sensitivity for higher boiling polar compounds but can decrease sensitivity for very volatile compounds. Sample matrix parameters such as ethanol concentration can also have different effects on the responses of different compounds. It is important to focus on the analytes of interest when optimizing sampling conditions, and to remain aware that conditions optimum for one set of compounds will not necessarily be optimum for another set of compounds.

Rocha and others [34] studied the behaviour of the SPME fibre (polyacrylate) regarding the different chemical classes of wine aroma compounds (monoterpenoids, aliphatic and aromatic alcohols, and esters). They analysed the extent of the changes in the concentration of one matrix component in the headspace equilibrium and, consequently, in the SPME sorption of the different matrix components. Relative response factors (rrfs), which establish the relationship between the concentration of the compound in the matrix liquid solution and the GC peak area, were estimated for all compounds. Quantification by SPME was shown to be highly dependent on the matrix composition; the compounds with higher "rrf" were the less affected. As a consequence, according to these authors, these limitations should be taken into consideration when using the data obtained with this methodology.

Liu and others [35] developed a new SPME coating made from butyl methacrylate (BMA), divinilbezene (DVB) and hydroxyl-terminated silicone oil (OH-TSO) with sol–gel and free radical polymerization; this was applied for simultaneous analysis of both polar and nopolar volatile compounds in wine. To check the matrix effects, various model wine matrices were investigated in detail. Matrix effects were compensated for by using the internal standard method and selecting the "volatile-free" wine as working standard. The method showed satisfactory linearity, precision, detection limits and accuracy.

The SPME was applied for the quantitative determination of eight aroma compounds present in a Portuguese muscatel wine must [36] and to determine aroma compounds of sparkling Cava wines (Certified Brand of Origin of Spain) [37]. In the latter they conclude that the quantities of ethyl decanoate, ethyl 2-decenoate, diethyl succinate, vitispirane, isoamyl hexanoate, isoamyl octanoate, and ethyl octanoate could be used as markers of the legal age limits of Cavas. The last three compounds also could be used to determine the approximate age of a sparkling wine.

Given that SPME is very appropriate for application in the field of volatile compounds, this technique is being widely used for the characterization of wines [38–47]. Bonino and others [45] utilized HS-SPME for the extraction of aroma compounds characterizing a Piedmont wine (Ruché) derived from a non-aromatic wine. It proved possible to identify a selection of 59 primary aromatic compounds, related to the typical flavour of Ruché. On the basis of experimental data obtained, the skin of the grape berries were attributed with primary importance as a source of varietal aromatic precursors, which are released easily during maceration in the presence of the enzymes cellulose and pectinase. The use of solid phase extraction-GC and SPME-GC has enabled the characterisation of the volatile composition of 23 monovarietal wines from 13 white and red grape varieties cultivated in the same area [6]. The chemometric analysis of these volatile compounds reveals that it is the terpenic compounds that are related most directly to the varietal aroma, among these, specifically terpineol, linalool and geraniol. The volatile compounds generated during the alcoholic fermentation present a relatively distinct composition depending on the type of fermentation carried out, and particularly according to whether maceration of the solid parts has been performed during the process. In the case of vinification without maceration, there is a relative increase of fatty acids and their ethyl esters, whereas in vinification with maceration, the ethyl esters of lactic, acetic and succinic acids are the compounds that are relatively more abundant. Marengo et al. [46] used SPME to analyse 68 samples of wines of Piedmont (Italy). A total of 35 analytes were identified; peak area data, corrected by internal standard, were used for pattern recognition treatments. The chemical characterization of the different wines was obtained by both supervized and unsupervized chemometric methods. Also, a HS-SPME-GC procedure was used to determine the composition of the volatile fraction of white wine samples from several Spanish certified brands of origin (CBO). According to the results obtained and by applying pattern-recognition procedures differentiation of the considered CBO was attained [47].

The technique using solid-phase microextraction-mass spectrometry-principal component analysis (HS-SPME-GC–MS-PCA) is proposed for the rapid distinction of wines based on the global volatile signature of the wine [48]. Principal component analysis (PCA) was applied to extract relevant chemical information by selecting the most significant mass fragments (m/z) that provide the better wine distinction. Rodriguez-Bencomo and others [49, 50] have studied the aromatic profile of sweet wines. For determination of esters, the PDMS-100 fibre, 40 min of extraction, headspace technique, stirring, saturation in sodium chloride and 16-ml vials were selected. Sugar content did not influence the extraction which allows this technique to be applied to sweet wine samples. However, for the determination of major compounds, the CW-DVB was selected. Applying a PCA to the results obtained enabled the wines to be differentiated according to the type of elaboration process employed.

A new-generation super elastic DVB/CAR/PDMS 50/30 μm fiber assembly was used for the headspace extraction of analytes from ice wine samples, for the determination of compounds with a wide range of polarities and volatilities [51, 52]. The profiles of volatile and semi-volatile compounds in various samples of Canadian and Czech ice wine were compared using PCA for the classification of the wines according to origin, grape variety, oak or stainless steel fermentation/ageing procedures used during the wine production or aroma profile differences between ice wines and late harvest wines.

In research on wine-making processes, Riu-Aumatell and others [53] studied the development of post-fermentation aroma and its evolution in cava wines during a long ageing time in contact with lees (more than 2 years). The extraction was performed with two alternative fibres, PDMS and the triple phase DVB–CAR–PDMS. Even though the volatile profile obtained by GC/MS was similar, the triple phase seems to be more suitable for monitoring the development of the volatile profile during ageing. Hexyl, 2-phenylethyl and isoamyl acetates significantly decrease over time, while 1,2-dihydro-1,1,6-trimethylnaphthalene (TDN), vitispirane and diethyl succinate significantly increase during ageing in contact with lees. Other authors have applied SPME to the monitoring of volatile compounds during wine fermentation [54–57].

The process of ageing in wines has also been studied using SPME. Carrillo and others [58] developed a HS-SPME-GC/MS method for the quantitative analysis in aged red wine of 14 volatile compounds from oak wood. These compounds are formed and extracted by wine when it is matured in oak barrels and are responsible for the particular organoleptic properties and high quality of these wines. The most important HS-SPME variables were optimized by experimental design technique in order to improve the extraction process. The selected conditions were as follows: 10 mL of sample in 20 mL sealed vials with addition of 30% of sodium chloride (saturated solution), DVB-CAR-PDMS fibre, 10 min of pre-incubation time, temperature of 70 °C, and 60 min of extraction time without agitation. In the same way, the SPME was used to determine the ability of caffeic acid and gallic acid to inhibit the decrease of volatile esters and terpenes during storage of a white wine and a model wine medium [59]. The HS-SPME was proposed for analysing the main volatile components from a sensory standpoint present in non-toasted and toasted oak wood of different origins [60]. The results obtained makes the proposed technique appropriate for its use in characterizing oak wood samples of different origins and in the selection of the most suitable oak wood to age wines and spirits, on the basis of the chemical composition of the wood samples. For the study of this type of compounds, Carrillo and Tena [61] developed a method for quantification by multiple headspace solid-phase microextraction (MHS-SPME). This technique is based on extrapolation to exhaustive extraction of the analytes from consecutive extractions (3 or 4) of the same sample. The method was applied in the analysis of volatile compounds in oak chips used to accelerate wine ageing as an alternative to traditional ageing in oak barrels [62].

The SPME has been compared with various other separation techniques. For example, Castro and others [63] compared it with rotary and continuous liquid–liquid extraction (LLE) and applied both techniques to the analysis of volatile compounds in “fino” sherry wine. The best conditions to extract these compounds using SPME and LLE were determined, and both methods were validated. No significant differences between results obtained by the two methods were found at a significance level of 5%. The LLE procedure is a method with high repeatability and offers the possibility of simultaneous extraction of several samples (up to 12); however the SPME technique is a solvent-free method presenting major advantages, such as small sample volume and higher sensitivity and simplicity. Bohlscheid and others [64] compared SPME with a SPE method using Amberlite XAD-2 resin for the extraction of volatile compounds. HS-SPME and XAD-2 performed similarly in the analysis of a Riesling wine; however, the HS-SPME method did not require organic solvents and was generally quicker to perform.

In respect of the use of SPME for the analysis of particular families of compounds in wines, its application to sulphurated compounds has been described. Volatile sulphur compounds play an important role in the aromat quality of foods and beverages. They are commonly found in foods that are in bad condition, giving them unpleasant flavour. From the enological point of view, these compounds, when present in wines are usually considered as off-flavours, and this means that the conditions under which the wines were produced were wrong. Mestres et al. [65] performed various studies in which they applied SPME for the analysis of these compounds in wines. HS-SPME has been combined with gas chromatography coupled to flame photometric detection (GC-FPD) to analyse volatile sulphides and disulphides. In one of the studies [66] it is concluded that the CAR-PDMS fibre was more efficient at extracting than other fibres like PDMS and PA, but its repeatability was worse. In another study [67] they analysed simultaneously sulphides and disulphides in wine aroma, applying a cryogenic trap to resolve the problems caused by poor desorption of the most volatile S-compounds. Later, they also analysed low-volatility organic sulphur compounds in wine by SPME [68, 69]. Similarly, Fang and Qian [70] developed a method for the analysis of these compounds based on SPME and gas chromatography-pulsed flame photometric detection. This technique has proved to be very sensitive

for sulphur compounds, and uses a pulsed flame, rather than a continuous flame as with traditional FPD, to achieve the generation of flame chemiluminescence. In the same way, 13 sulphur compounds, usually considered as possible off-flavouring volatiles, were quantified by a HS-SPME method on 80 not off-flavouring wines of four varieties and of five vintages produced in the North Italian Trentino region [71]. Also López and others [72] developed a method based on the automated HS-SPME sampling of small volumes of wine with CAR-PDMS fibers and subsequent GC-PFPD for the quantitative determination of highly volatile sulfur compounds present in wine. This method less sensitive to matrix effects.

In the case of volatile phenols, Martorel et al. [73] developed a method of analysis of ethylphenols in red wine utilizing SPME. The fibres used were coated with 100 µm of polydimethylsiloxane (PDMS). Castro and others [74] optimized a SPME method for ethylphenols and vinylphenols in white and red wines. Silica fibre coated with Carbowax-divinylbenzene (CW-DVB) was found to be more efficient at extracting these compounds, Fig. 4.

Methoxypyrazines are extremely potent odorants found in vegetables such as bell peppers and french beans and is an important wine grape flavour compound in varieties such as Cabernet Sauvignon and Sauvignon blanc. They have a vegetative, herbaceous odour, similar to bellpepper, and a very low sensory threshold of between 1 and 10 ng/L in water. Sala et al. [75] have developed a method for determining 3-alkyl-2-methoxypyrazines in musts by means of gas chromatography with nitrogen–phosphorous detection (GC-NPD) with HS-SPME. It provides high recoveries, detection limits at the 0.1–1 ng/L level and a linearity range of 2–100 ng/L. The method has been applied to experimental musts of Cabernet Sauvignon and Merlot, and analytes have been monitored during the ripening and at harvest. In another study, these authors utilized the same technique after a sample clean-up by distillation to remove ethanol and other volatile compounds that could interfere in the SPME [76]. SPME with stable isotope dilution has been

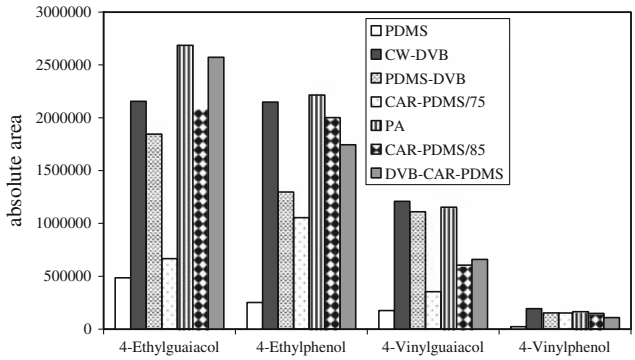

Fig. 4 Fibre Screening. Peak areas (mean values) obtained for each volatile phenol

applied for quantification of metoxypyrazines in wines [77, 78]. Ryan and others [79] optimized a HS-SPME method for the determination of methoxypyrazines in wine. Analysis was performed using comprehensive two-dimensional gas chromatography with novel detection capabilities, including nitrogen phosphorus detection (GCxGC-NPD) and time-of-flight mass spectrometry (GCxGC-TOFMS). In the latter, stable isotope dilution was performed for the quantitation of 2-methoxy-3-(2-methylpropyl) pyrazine (IBMP), using labelled 2-(2H3)methoxy-3-(2-methylpropyl)pyrazine as the internal standard, and resolution of the two analogues was facilitated using the deconvolution capabilities of the TOFMS. Both the techniques were highly sensitive, yielding detection limits for IBMP of 0.5 and 1.95 ng/L, respectively. Quantitation of IBMP in a Sauvignon blanc wine by the two techniques provided comparable concentrations indicating that the HS-SPME method was unaffected by wine matrix effects.

In respect of cork taint compounds analysis, there are several examples of the use of SPME for this purpose [80–98]. Martínez-Uruñuela et al. [89, 90] optimized an acetylation reaction for the derivatisation of chlorophenols using a Doehlert design for direct application in wine samples. The final objective of this reaction is to transform chlorophenols into less polar compounds thus improving their chromatographic performance. In the derivatisation reactions, chlorophenols can be transformed into the corresponding esters using acetic anhydride. Also Pizarro et al. [91] use a headspace solid-phase microextraction with on-fiber derivatisation method for the direct determination of haloanisoles and halophenols in wine. Insa et al. [92] developed and applied two methodologies based on SPE and SPME coupled to GC-electron-capture detection to determine TCA, TeCA and PCA in cork macerate and wine samples. For this purpose, a C18 cartridge and a styrene-divinilbenzene-based sorbent were evaluated under different experimental conditions. In addition, a SPME method using a PDMS fibre was optimized to achieve the best extraction conditions for the chlorophenolic compounds. The same authors [93] did a comparative study to check the matrix effect on the extraction of three chlorophenols in synthetic and commercial wines (white and red wines). The matrix effect also has been studied by Vlachos et al. [94] and Pizarro et al. [95] that were identified causing significant bias to the quantitative analysis. Gómez-Ariza et al. [96] compared the multiple HS-SPME and GC-MS with several analytical approaches based on pervaporation, an innovative membrane-based technique similar to dynamic headspace. In the pervaporation approach the introduction of the membrane contributes to the matrix elimination before the sorption step; in MHS, the multiple extractions involved in the process reduce the matrix effect. In 2006, Riu et al. [97] developed a method for quantifying the total endogenous chloroanisoles in the differ-

ent kinds of cork by using a 50/30 μm stableflex divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre and GC with electron capture detector. This method was applied to analyse the total amount of chloroanisoles in different kinds of cork stoppers (natural, agglomerated and sparkling wine stoppers) [98].

Also the SPME has been used to quantify by internal standardization 18 molecules that are of interest because they have been identified as being responsible for a large number of faults encountered in today's wine industry [99]. In the same way, a method was developed for the simultaneous analysis of volatile compounds responsible for off-flavours in cork-stoppered wines [100].

Another important aspect of the use of SPME for volatiles in wines is its application to the determination of terpenes and similar compounds. Peña et al. [101] developed a method for the analysis of certain terpenes in wine samples using SPME and GC-MS. The best results were obtained by direct immersion of the fibre using a sampling period of 15 min with constant magnetic stirring. Later, these authors [102] compared the method with ultrasound-assisted extraction (UAE). The application of both methods to red wine samples showed that UAE provides better extraction of monoterpenic compounds than SPME, although SPME remains an attractive alternative technique due to its speed, low sample volume requirements and solvent free character. Camara and others [103] developed a method based on HS-SPME for the determination of trace levels of terpenoid compounds in samples of must and Madeira wine, a liquorous wine with an alcoholic content of 18% (v/v). The best results were obtained using a 85-μm polyacrylate fibre, with a 60-min headspace for must and 120 min for wine samples. Thirty-six Madeira wine samples from white grape varieties were analysed in order to estimate the free fraction of monoterpenols and C13 norisoprenoids [104].

In addition to utilizing SPME for the analysis of families of compounds, SPME is also used for the analysis of specific individual compounds in wine that have a particular importance, for several reasons, in the control of wine-making processes. Hayasaka and Bartowsky [105] developed a method to analyse diacetyl, one of the important flavour compounds in dairy products as well as wine. The presence of diacetyl in wines is usually associated with malolactic fermentation, a process which can occur simultaneously with or following the alcoholic fermentation.

In respect of the analysis of vinegar, Castro et al. [106, 107] applied HS-SPME to the analysis of aroma compounds in this enological product. Silica fibre coated with CAR-PDMS was found to be more efficient than other fibres at extracting these compounds. The extraction efficiency was inversely affected by the acetic acid content—an increase in the acetic acid concentration decreases the extraction efficiency. No interference is observed with the increase in content of polyphenols. The method was applied to various Sherry wine vinegars.

As important as obtaining a specific quality of a vinegar is the need to determine objectively the appropriate parameters that allow us to characterize and differentiate one vinegar from another. With this objetive, Natera et al. [1] developed several pattern recognition approaches that permit classification of vinegar samples according to raw material (wine, cider, alcohol, etc.) and production process, using different analytical parameters, such as polyphenolic content, organic acids, and volatile compounds. Volatile compounds were determined by SPME and GC. Also Pizarro et al. [108] use the volatile compounds to classify different vinegar types. The collected chromatographic signals were analysed using the stepwise linear discriminant analysis method, thus simultaneously performing feature selection and classification.

Cocchi and others [109] applied a feature selection and classification algorithm based on wavelet packet transform to the discrimination of balsamic vinegars. All the samples have been characterized on the basis of the gas chromatographic profiles of the headspace volatile fraction, sampled by SPME.

Furan derivatives are characteristic of the caramel-like flavour of Italian traditional balsamic vinegar. They are formed with Maillard reactions (or non-enzymatic browning) between reducing sugars and amino acid that occur during the cooking of the grape must and during the ageing period. The presence of 2-furfural and 5-methylfurfural in balsamic vinegar is normal and some producers are interested in determination of the level of these molecules in the finished product in order to evaluate organoleptic properties and detect possible commercial frauds. Giordano and others [110] developed a method for the determination of 2-furfural and 5-methylfurfural in vinegar, with HS-SPME coupled to GC–MS. A DVB-CAR-PDMS fibre was used and SPME conditions were optimized, studying ionic strength effect, temperature effect and adsorption time. Isotope dilution calibration was performed, using 2-furfural-d4, so avoiding the standard additions method.

The SPME has also been applied to the study of the volatile compounds of brandy. Brandy aroma is influenced by the environment in which the grapes are grown, grape variety, grape maturity, fermentation processes, distillation conditions and, most importantly, the duration of the ageing process and ageing temperature, as well as by the use of oak barrels and the relative humidity in the barrel house. Ebeler et al. [111] utilized SPME to analyse the volatile composition of brandy, comparing the results to a traditional continuous liquid-liquid extraction procedure. In addition, a combination of SPME with GC-olfactometry was used to provide more detailed information on sensory characteristics of varietal brandies.

Watts and Butzke [112] applied SPME for quantifying methylketone concentrations in a large number of commercially available Cognacs to find possible correlations with other volatiles and to confirm the suitability of methylketones as an impact compound group to assess the quality of premium brandies. Soon after, these same authors [113] utilized a method of SPME and GC-MS and partial least-squares (PLS) regression to predict sample age and to separate Cognacs of different ages using only chromatographic peak areas. The subset consisting of 17 volatiles (13 ethyl esters and 4 methyl ketones) could predict sample age with a high degree of accuracy.

Also the SPME has been applied for the determination of volatile and semi-volatile compounds from different alcoholic beverages: beer and whisky [114].

SBSE

The technique termed sorptive extraction using a stir bar agitator (SBSE) is based, like SPME, on the use of an apolar sorbent polymer, polydimethylsiloxane (PDMS), as the medium of extraction of analytes in liquid and gaseous samples. PDMS presents a series of characteristics that have made it the sorbent material most commonly used for this type of technique. These include, in particular, its inert character, which reduces the risk that compounds may be generated on its surface; the relative ease with which it can be synthesized, and therefore the inevitable differences between the various manufacturers in respect of consistency and reproducibility are minimized; and the degradation products are fairly easy to identify by mass spectrometry.

SBSE is not the first technique to be based on the use of PDMS as extraction medium. Before SBSE appeared, researchers developed various other techniques, some of which will be mentioned next. The first to be developed was open tubular trapping (OTT) [115]. This technique utilizes a type of open capillary column, with PDMS on its internal wall. Subsequently the previously mentioned SPME [116] was developed; this method, as already explained, operates through the PDMS available on the surface of the needle of a device similar to a syringe. And later, but prior to SBSE, the gum-phase extraction (GPE) was developed, based on the use of packed beds formed from particles of 100% PDMS [117].

Finally, at the beginning of the 1990s, a method of extraction by sorption using a stir bar was developed (SBSE); this method offered the sensitivity of the PDMS packed beds, and the range of application (in terms of volatility) of SPME [118]. A stir bar was incorporated into a glass tube with an external diameter of 1.2 mm, and coated with a 1-mm thick layer of PDMS, providing a total width to the stir bar of 3.2 mm external diameter. The stir bars are introduced into the aqueous samples and the extraction takes place through the agitation or stirring generated by the bar within the liquid. The quantity of PDMS can vary with the length, which is typically from 10 mm (55 μL of PDMS) to 40 mm (219 μL of PDMS), which are applied, respectively, to small and large volumes. After a specified time of agitation/stirring, the bar is removed from the sample, is placed inside a glass tube, and is then transferred to a thermal desorption instrument (TDS), where the analytes are thermally removed from the bar. Subsequently a process of cryoconcentration takes place (normally with liquid nitrogen), so that the analytes are concentrated again before entering the chromatograph. Using this procedure much narrower analytical peaks are obtained. Finally, and after the application of heat, the analytes are transferred, in the majority of cases, to a gas chromatograph coupled to detection by mass spectrometry.

The SBSE presents a series of clear advantages over the rest of the extractive techniques described in this review. For a start, this technique is solvent-free, unlike SPE. This brings various additional advantages: the samples are not in contact with any solvent, and so are less likely to be altered by contamination or the formation of artefacts during the extraction process. For this same reason, the technique is much friendlier to the natural environment, since it does not generate residuals of any kind. Another advantage of this technique is that it can be almost completely automated thus making it very simple and fast to apply repeatedly. The technique requires almost no handling of the sample on the part of the analyst, nor does it require prior treatment of the sample. This means that the possibility of analytical error is considerably reduced. Compared with SPME, SBSE provides greater analytical sensitivity: it reaches much lower detection and quantification limits. The reason for this is that, in SBSE, the quantity of PDMS employed is rather greater, with the result that the extractive capacity is also greater.

Against this, and due also to the use of the PDMS, SBSE presents a clear disadvantage compared with other extractive techniques. This is the limited extraction capacity of PDMS for polar substances, given its marked apolar character, and PDMS is the only sorbent utilized to date in SBSE. This problem is palliated to some extent since the quantity of PDMS used in the technique is relatively large, and it is due to this that PDMS manages also to extract substances of polar character, although less efficiently. Another disadvantage is that, due to the nature of the technique, a prior process of optimization is required, both of the extraction conditions (extraction time, speed of stirring, temperature, etc.) and of the conditions of desorption (temperature of desorption, time of desorption, temperature of cryoconcentration, etc.) for each specific case.

The effect of this is that the technique cannot be immediately applied to a case that has not been previously studied.

Application of SBSE to enological products

Most of the applications of this technique to the determination of volatile compounds in enological products are focused on wine as the object of analysis, including both white and red wine, and wine of different Denominations of Origin. However, in addition to wine, there are also studies that make reference to vinegar, grapes and must.

Due to their negative influence on the flavour, the analysis of TCA in wines using SBSE has been studied by several authors [119, 122]. In a study of Zalacaín et al. [121], not only was the presence of 2,4,6-trichloroanisole (TCA) detected, but a method of analysis was also developed that dispensed with the preconcentration stage and offered a relatively short time for analysis (2 h), for the detection and quantification of TCA 2,3,4,5-tetrachloroanisole, (TeCA), 2,3,4,5,6-pentachloroanisole (PCA) and their respective phenols in samples of red and white wine. The optimized extraction conditions were 10 ml of sample, stirred at 700 rpm for one hour, at ambient temperature, and adjusting the pH to 3.6. The SIM mode of quantification was also utilized. Lower values of detection and quantification than any that had previously been obtained were achieved, with concentrations lower than their olfactory threshold values. Another recent study by Lorenzo et al. [122] dealt with identifying the presence of halophenols and haloanisoles in enological products by means of SBSE; these compounds are specifically derived from cork stoppers used for the bottling of wines. In this case the technique known as headspace sorptive extraction (HSSE) was employed as an extension of SBSE; this was developed by Tiepont et al. [123]. This variation is based on inserting the stir bar only into the headspace of the solution to be analysed, not in the actual liquid, but the analytes are retained by the PDMS in the same way, Fig. 5. For the optimization of the extraction conditions, the authors injected a solution of TCA into synthetic wine, on the end of a cork stopper. It was found that the best results were obtained after submitting the spiked corks to 100 °C for 1 h, followed by a stabilisation time of 30 minutes at room temperature. The method presented good linearity in the range of 1–70 ng/g, and coefficients of correlation of 0.90 and 0.99; reproducibility and repeatability values were also acceptable. In relation to the analysis of chloroanisoles and chlorophenols in enological products, it has also been applied directly to cork material [124].

The analysis of volatile compounds in wine by means of SBSE, has been studied by several ways. It has been applied to wines aged in oak wood casks [125], to study the wine primary aroma compounds [126] or to study the possible effect of grape maturity on wine aroma [127]. Díez et al.

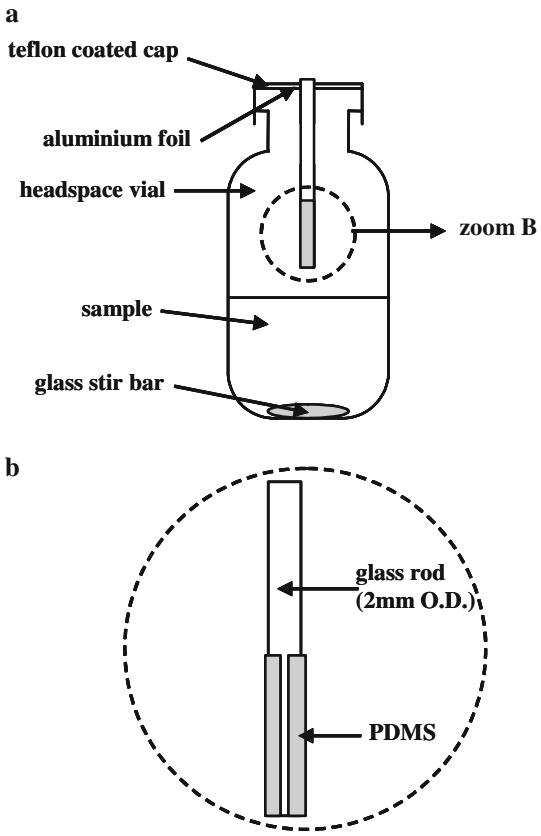

Fig. 5 Headspace sorptive extraction (*HSSE*) sampling setup: headspace vial (**a**), and detail of the HSSE-PDMS bar (**b**)

[128] devized a method of analysis using stir bar sorptive extraction for the quantification of the volatile phenols 4-ethylphenol, 4-ethylguaiacol, 4-vinylphenol and 4-vinylguaiacol. The following conditions of extraction were optimized for this case: the dilution of the sample was 1:4; the sample volume was set at 15 ml of diluted wine; extraction time was 60 min; the speed of stirring was 900 rpm; and NaCl was not added to the samples. The method was validated analytically, and good values, not only of r^2 (higher than 0.9964), but also of linearity, RSD, recovery, sensitivity/detection limits and quantification were obtained. Unlike the preceding case, here the SIM mode was used exclusively for the quantification, and this contributed to lowering the detection limits. The method optimized was applied to various samples of red and white wines and fino sherry.

The SBSE has also been used jointly with SPME. Alves and others characterized the aromatic profile of Madeira wine, using these two techniques, both coupled to GC/MS [129]. While SPME was used for identifying the majority components of the aroma of Madeira wine, SBSE, being more sensitive, was used for the determination of the minority components of the aroma, which play a very important role in the aromatic complexity of this wine and

are most important for the bouquet. Among the families of compounds that were studied by SBSE, we find esters, carboxylic acids, alcohols, aldehydes, pyrans, lactones, monoterpenes, sesquiterpenes and C13 norisoprenoids. The authors found that there was an excellent correlation between the length of ageing of the wine and the abundance of the compound designated *cis*-oak lactone; hence it was concluded that this compound is a valuable descriptor for characterising aged Madeira wines, in addition to it contributing to the aroma of the wine. The results obtained by SBSE allowed different types of wine from Madeira to be differentiated by employing the wines' content in volatile compounds.

Komes et al. [130] carried out a comparative study to determine which extraction technique was best to replace the commonly-used liquid-liquid extraction with 1,1,1-trichlorofluoromethane, for isolating the volatile compounds of white wine prior to their analysis by gas chromatography. SPME and SBSE, as well as liquid–liquid extraction, were compared. The authors found that, although both SBSE and SPME offered the possibility of automating the procedure, using a small sample volume, and a rapid and simple handling, the recovery of the aromatic compounds was restricted, given the properties of discrimination of the polymeric phase. However, the results obtained by SBSE were more similar to those by liquid-liquid extraction than those obtained by SPME.

Kittel and others [131, 132] used SBSE coupled to gas chromatography/olfactometry (GC/O) to study a series of wines in which unwanted changed related to the aroma had been detected. The "untypical" ageing aroma (UTA), as it is commonly called, is produced due to oxidative changes in the aromatic compounds of the wine, and it is very common to find it in various types of European wine. Thanks to the application of SBSE, the authors reached the conclusion that, although UTA could occur in American wines, it was not due to the presence of the compounds that were thought to be responsible, since these were found below the limit of detection of the technique.

The study of preservative compounds in various food samples including white and red wines and balsamic vinegars have also been performed using SBSE [133]. With reference to vinegar as the exclusive object of study, Pfannkoch and Whitecavage [134] applied SBSE/GC/MS to the determination of the particular volatile compounds present in the aroma of balsamic vinegar, among other matrices. By using this technique, the interferences and problems derived from the presence of the more polar components of the matrix were eliminated.

In 2006, a new application of SBSE related to vinegars has appeared in the bibliography; Durán Guerrero et al. [135] have proposed and evaluated a method for the analysis of volatile compounds in vinegars. Using chemometric tools, the authors optimized the analytical conditions of both extraction and desorption. They conclude that the best analytical conditions of extraction are 25 ml of sample without dilution, stirring for 120 min at 1,250 rpm, and the addition to each sample of 5.85 g of NaCl. The optimum analytical conditions of desorption are as follows: a desorption temperature of 300°C, maintaining this maximum temperature for 10 min, a flow of helium of 75 ml/min during this process, and a temperature of −140 °C in the subsequent cryoconcentration. Using this method adequate values of linear range and of detection limits were obtained for the study of the volatile compounds present in vinegar. In a later work [136], the authors validated the method and compared this methodology with another one based on SPME [106, 107]. The amount of studied compounds was increased in this second study. Lower detection and quantitation limits and better repeatability and reproducibility values were obtained for SBSE. Also better sensitivity was observed for SBSE, due to its greater capacity of extraction, Fig. 6.

Focusing their study on the volatile compounds present in the original grapes, Salinas et al. applied stir bar sorptive extraction to the evolution of the volatile compounds during the maturation of grapes of the Monastrell variety [137]. Luan et al. [138] applied the technique to different varieties of grape during their maturation, for the analysis of monoterpenes (more specifically, metabolites of linalol and of citronellol). These authors made use of SBSE for the analysis of those compounds that had the lowest concentration (of the order of ppb), while for the rest of the compounds, they used solid phase extraction as the method of isolation. Because there were stereoisomers among the analytes studied by the authors, the technique known as enantioselective-multidimensional gas chromatography–mass spectrometry was used, coupled to the extraction procedures previously described. With this technique the authors managed to separate the stereoisomers of the metabolites studied, using for this a second chiral column with a modified cyclodextrin as stationary phase, Fig. 7. This enabled them to determine the stereoisomeric ratios of the analytes studied, which can be of interest for monitoring the authenticity of the aroma of the grape or wine. This is the first reference found in the bibliography where SBSE is used coupled to a multidimensional chromatographic system; this system has enabled the elucidation of two different reaction routes for the formation of several key analytes in the maturation of the grape.

A new study dealing with SBSE and volatile compounds in enological products, carried out by Caven–Quantrill and Buglass [139] has been published. The authors compare the SBSE technique with the traditional micro-scale simultaneous distillation-extraction (SDE) for the analysis of volatile compounds in grape must. The conditions of extraction

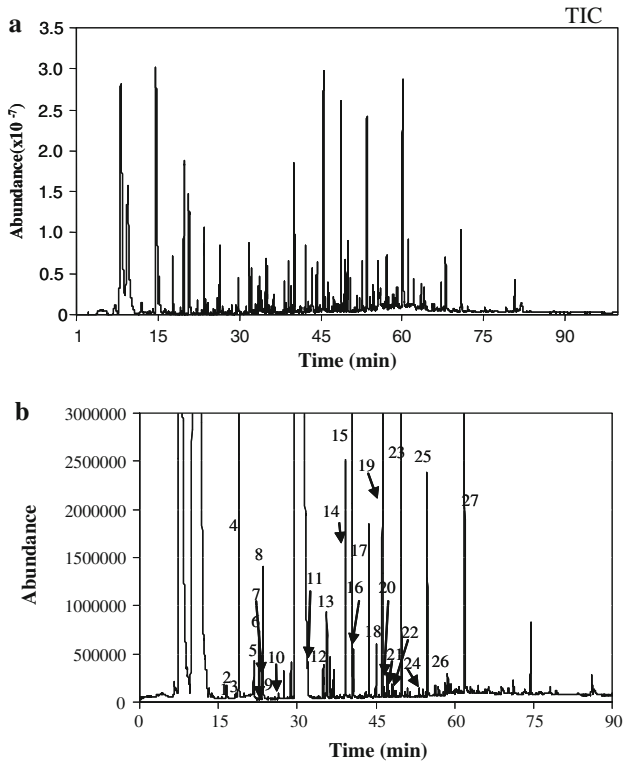

Fig. 6 Total ion chromatogram obtained for a vinegar sample by means of SBSE and SPME. **a** SBSE Retention times (min): Ethyl isobutyrate (13.62); propyl acetate (13.99); isobutyl acetate (15.76); ethyl butyrate (16.84); *n*-butyl acetate (18.38); ethyl isopentanoate (18.46); hexanal (18.70); isobutanol (19.71); isopentyl acetate (20.57); ethyl pentanoate (20.77); 1-butanol (21.84); *trans*-2-hexenal (24.01); isoamyl alcohol (23.84); 2-methyl-l-butanol (24.12); ethyl hexanoate (24.65); hexyl acetate (25.80); 3-hydroxy-2-butanone (26.62); *cis* 3-hexenyl acetate (27.59); ethyl lactate (28.51); hexan-l-ol (28.87); *cis*-3-hexen-1-ol (30.04); *trans*-2-hexen-l-ol (30.82); ethyl octanoate (31.87); 2-furaldehyde (32.87); benzaldehyde (35.15); isobutyric acid (36.84); 5-methyl-2-furaldehyde (36.95); 2-acetyl-5-methylfuran (38.54); butyric acid (38.89); isovaleric acid (40.28); diethyl succinate (40.58); α-terpineol (41.51); benzyl acetate (42.64); ethyl-2-phenyl acetate (44.59); phenylethyl acetate (45.95); hexanoic acid (46.57); benzyl alcohol (47.03); 2-phenylethanol (49.21); 2-ethyl hexanoic acid (50.17); 4-ethylguaiacol (52.87); octanoic acid (53.75); eugenol (57.21); 4-ethylphenol (57.36); 5-acetoxymethyl-2-furaldehyde (58.00); decanoic acid (60.39); diethyl fialate (63.87); 5-hydroxymethyl-2-furaldehyde (68.90). **b** SPME *1* *n*-butyl acetate; *2* ethyl pentanoate; *3* 2-methyl-1-propanol; *4* isoamyl acetate; *5* 4-methyl-2-pentanol (IS); *6* ethyl hexanoate; *7* 2-methyl-1-butanol; *8* isoamyl alcohol; *9* hexyl acetate; *10* 3-hydroxy-2-butanone; *11* 2-furancarboxaldehyde; *12* benzaldehyde; *13* 2,3-butanediol; *14* ethyl decanoate; *15* isovaleric acid; *16* diethyl succinate; *17* 1,1,6 trimethyl-1,2-dihydronaphtalene; *18* ethyl-2-phenyl acetate; *19* phenylethyl acetate; *20* hexanoic acid; *21* α-ionona; *22* benzyl alcohol; *23* 2-phenylethanol; *24* 4-ethylguaiacol; *25* octanoic acid; *26* 4-ethylphenol; *27* decanoic acid

and desorption for the SBSE method, optimized using a synthetic must were the following: for the extraction a stirring time of 2 h at 1,000 rpm was used; for the desorption process, on the one hand, a final desorption temperature of 300 °C (for 5 min) and a helium flow of 70 ml/min were utilized, and on the other, in the cryogenic trap system, the

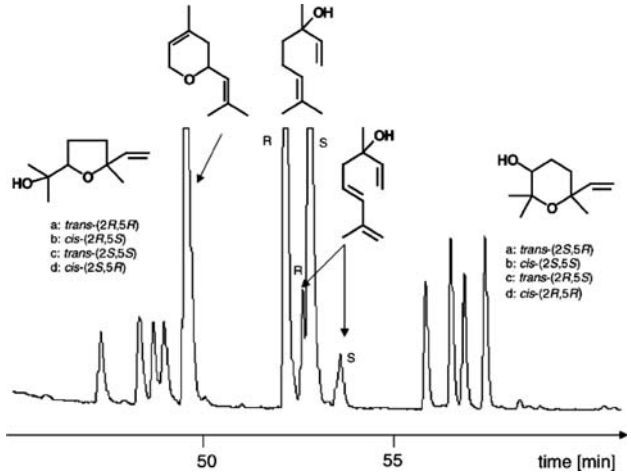

Fig. 7 Second dimension column chromatogram illustrating the separation of a standard mixture (in the order of their elution times): furanoid linalool oxide, nerol oxide, linalool, hotrienol, pyranoid linalool oxide. (Reprinted from [138] with permission from Elsevier)

cryoconcentration temperature was −50 °C and the final temperature was 260 °C (for 20 min). When they compared the two methods, the authors found that SBSE gave lower recovery and reproducibility values than SDE; however, the first of the methods was rather more sensitive, with 126 volatile compounds being identified in samples of real must, against 98 compounds identified using SDE; SBSE also presented the advantages already known, such as the rapidity and simplicity of the analysis, the scope for being automated, and the fact that solvents are not needed.

The following table, Table 2, shows some of the analytical conditions employed by various authors for the analysis of volatile compounds in the different matrices studied.

SPDE

The origin of SPDE lies in various attempts made to overcome some of the disadvantages of SPME, such as the fragility of the fused silica, unprotected stationary phase coating and limited film thickness.

In 2000, Chromtech (Idstein, Germany) commercialized an inside-needle technique known as solid-phase dynamic extraction (SPDE). It is also known as the "magic needle". In this headspace technique, the analytes are concentrated on a film of different coatings onto the inside of a stainless steel needle of a gas syringe (Fig. 8). Different commercially available SPDE needle coatings with different polarities can be found: polar polyethylene glycol WAX phase (50 μm film thickness and 56 mm film length), cyanopropylphenyl/polydimethylsiloxane phase (50 μm film thickness and 56 mm film length), non-polar polydimethylsiloxane phase (PDMS, 50 μm film thickness and 56 mm film length) and polydimethylsiloxane with 10%

Table 2 SBSE analytical conditions used to analyse volatile compounds in enological products

Authors	Matrix	Analytes	Extraction analytical conditions
Sponholz et al. [119]	Wine	2,4,6-Trichloroanisole	–
Hayasaka et al. [120]	Wine	2,4,5-Trichloroanisole; flavour; agrochemicals.	*Sample volume*: 10 ml; *stirring speed*: 700 rpm; *extraction time:* 90 min.; *extraction temperature*: 25 °C
Zalacain et al. [121]	Wine	Chloroanisoles	*Sample volume*: 10 ml; *stirring speed*: 700 rpm; *extraction time:* 60 min.; *extraction temperature*: room temperature; pH: 3.6
Lorenzo et al. [122] (HSSE)	Cork stoppers	Halophenols and haloanisoles	*Extraction temperature*: 100°C; *extraction time:* 60 min.; *stabilization time*: 30 min.
Callejón et al. [124]	Cork stoppers	Chloroanisoles and chlorophenols	*Previous extraction:* 24 hours using 75%-ethanol/25%-water. *Sample volume*: 1 ml of diluted extract; *stirring speed*: 700 rpm; *sample dilution:* 1:10; *extraction time:* 60 min; *pH*: 3.6
Marín et al. [125]	Oak-aged wine	Flavour	*Sample volume*: 25 ml; *stirring speed*: 700 rpm; *extraction time*: 90 min; *extraction temperature*: room temperature.
Zalacain et al. [126]	Wine	Flavour	*Sample volume*: 25 ml; *stirring speed*: 700 rpm; *extraction temperature*: 60 °C; *extraction time:* 90 min
Fang et al. [127]	Wine	Flavour	*Sample volume*: 10 ml of diluted wine; *stirring speed*: 1000 rpm; *sample dilution*: 1:2; *extraction time:* 12 hours; NaCl addition
Díez et al. [128]	Wine	Volatile phenols	*Sample volume*: 15 ml of diluted wine; *stirring speed*: 900 rpm; *sample dilution*: 1:4; *extraction time:* 60 min
Alves et al. [129]	Wine	Flavour	*Sample volume*: 10 ml of diluted wine; *stirring speed*: 800 rpm; *extraction temperature:* 20 °C; *sample dilution*: 1:2; *extraction time:* 60 min
Komes et al. [130]	Wine	Flavour	–
Kittel et al. [131, 132]	Wine	Off-aromas	–
Ochiai et al. [133]	Wine and balsamic vinegar	Preservatives	*Sample volume*: 10 ml; *stirring speed*: 1000 rpm; *extraction temperature*: 25°C; *extraction time:* 120 min.; pH: 1.5; NaCl addition
Pfannkoch et al. [134]	Balsamic vinegar	Flavour	–
Duran Guerrero et al. [135, 136]	Vinegar	Flavour	*Sample volume*: 25 ml; *stirring speed*: 1250 rpm; *extraction time*: 120 min.; NaCl addition.
Salinas et al. [137]	Grapes	Flavour	•Maceration. *Sample amount*: 200 g.; Maceration time: 120 min •Extraction. *Sample volume*: 100 ml of diluted crushed grape; *stirring speed*: 700 rpm; *sample dilution:* to 1 L.; *extraction time*: 360 min.
Luan et al. [138]	Grapes	Monoterpenes	*Stirring speed*: 450 rpm; *extraction temperature*: room temperature; *extraction time*: 30 min.; pH: 4
Caven-Quantrill and Buglass [139]	Grape juice	Flavour	*Sample volume*: 20 ml; *stirring speed:* 1,000 rpm; *extraction temperature*: room temperature; *extraction time*: 120 min

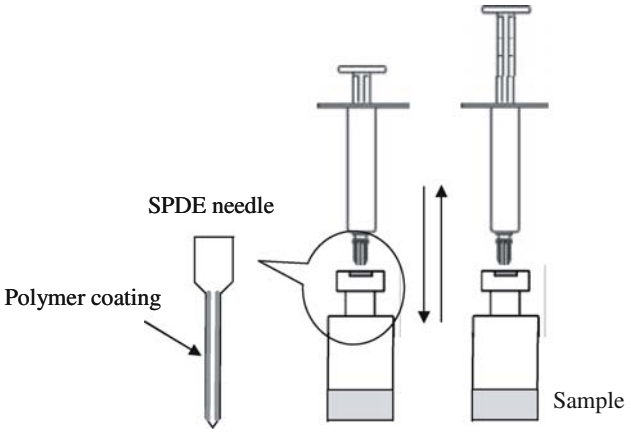

Fig. 8 SPDE sampling device

embedded activated carbon phase (PDMS/AC, 50 µm film thickness and 56 mm film length).

The volume of coatings on the SPDE needle wall is about 4.5 µl in comparison with about 0.6 µl for a SPME fibre. Thanks to this volume, SPDE achieves a higher concentration capability.

Analytes are accumulated in the polymer coating by pulling in and pushing out a fixed volume of the headspace of the sample for a predetermined number of times. Thus SPDE operates under dynamic conditions, keeping constant the headspace volume. Then analytes are thermally desorbed by a flow of helium or nitrogen into the GC injector.

The main difference between SPDE and SPME is that the first is a non-equilibrium sampling method. SPDE must therefore be carried out under rigorous standard conditions in order to obtain reliable results, in particular for quantitative analysis.

A review discussing the technological aspects and some general applications of this technique has been lately published [140].

Application of SPDE to enological products

To date, SPDE has been applied to different analytes in diverse matrices [141–146]. Bicchi et al. [141] applied this technique to analyse the volatile fraction of different food matrices: rosemary leaves, coffee, banana, and red and white wines. In this study, several parameters such as sampling temperature, number of aspiration cycles, plunger speed and aspired volume for each cycle, helium desorption volume and desorption plunger speed, were evaluated. For extraction, all samples were maintained at 50 °C for 15 min, except for banana, which was sampled at 35 °C. The number of aspiration cycles was established at 50, whereas the aspiration plunger speed was fixed at 50 µL/s. A total of 1 ml of helium was employed for desorption. The plunger speed for desorption was 15 µL/s. These last two parameters were carefully studied because they influence recovery. A low plunger speed is necessary for the complete desorption of the analytes into the helium stream. It involves a higher desorption time and a low initial oven temperature to concentrate the desorbed analytes. These authors used 20 °C as initial oven temperature for all matrices, with the exception of banana (0 °C). Under these conditions, good repeatability with RSD values lower than those obtained using SPME was obtained.

Jochmann et al. [147], after the optimization of certain extraction parameters have applied SPDE for the determination of fusel oils in alcoholic beverages such as beer, wine, brandy and rum. The extraction temperature was set as 70 °C. All analytes showed stable responses after 50 extraction cycles and the addition of salt produced significantly higher extraction efficiencies. Using these conditions and a WAX phase, the RSD values ranged from 2 to 14%. The polar WAX phase and non-polar polydimethylsiloxane with embedded activated carbon were more efficient for the extraction of fusel oils. Taking into account the high sensitivity of the technique, the authors indicate that the samples can be diluted to suppress a possible matrix effect.

In general, a significant advantage of SPDE over SPME is the robustness of the capillary, together with a higher sensitivity of this technique.

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