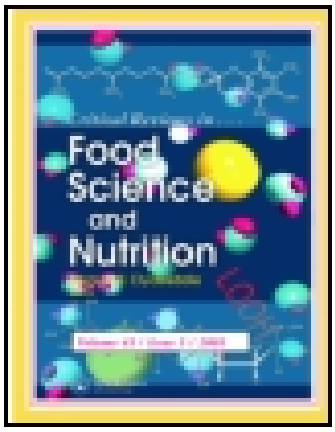


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# Headspace solid-phase microextraction for wine volatile analysis

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## 1- Introduction

Wine is a complex beverage matrix which contains over 800 identified volatile compounds (Noguerol-Pato et al., 2009). Several studies based on solid-phase microextraction for extraction and concentration of analytes have been conducted, covering different aspects in the field of wine, such as origin characterization and flavor analysis (Castro et al., 2008a, Pozo-Bayón and Reineccius, 2009, Noguerol-Pato et al., 2009, Tao and Zhang, 2010), origin comparison (Sagratini et al., 2012, Câmara et al., 2007, Setkova et al., 2007), and off- flavor identification (Vlachos et al., 2007, Pizarro et al., 2007a, Kotseridis et al., 2008, Pizarro et al., 2008).

The presence or absence of aroma compounds plays a vital role in the quality of food and beverages. In the case of wine, aroma properties have a significant influence on the acceptance and appreciation of the product (King et al., 2010, Corduas et al., 2013, Bindon et al., 2014).

Several extraction methods have been used prior to the analysis of volatile compounds in wines, such as distillation, solvent extraction and solid-phase extraction. Compared to these conventional extraction techniques, solid-phase microextraction (SPME) has the advantages of being simple, fast, inexpensive, no-solvent requirements and limited manipulation of the sample (Sagratini et al., 2012). Furthermore, headspace SPME (HS-SPME) has been widely applied in wine analysis (Rebière et al., 2010, Noguerol-Pato et al., 2009, Jeleń and Szczurek, 2010, Weldegergis et al., 2011).

 

This article will review available theoretical and empirical studies concerning solid-phase microextraction and headspace solid-phase microextraction in the study of some defaults and volatiles characteristics in wine, and their use for the extraction of volatile compounds.

### 1.1 Solid-phase microextraction : overview

Headspace solid-phase microextraction (HS-SPME) is a solvent-free procedure for sample preparation that can be easily coupled to gas chromatography (GC) and is fast, economical, suitable for direct analysis of complex matrices, and provides lower detection limits than other headspace techniques (Carrillo et al., 2006).

The interest of HS-SPME was quickly recognized, illustrated by the linear increase in the number of publications as shown in figure 1. The triangles show the number of articles published related to SPME, and the squares, those related to the SPME applications in wine, based on searching the *Scopus* database, between 1998 and 2011, using the following keywords : HS-SPME / WINE / AROMA / FLAVOR.

## 2- Solid – phase microextraction: theory and basic principles

The basic principle of the SPME approach is to use a small amount of the extracting phase.

The configuration of SPME uses a small fused silica fibre, coated with a polymeric phase, which is mounted in a syringe-like device. The analytes are absorbed, or adsorbed, by the fibre phase until the system reaches equilibrium. When an equilibrium distribution

between the matrix and fibre coating is reached, the extraction is considered to be complete. The equilibrium condition can be described as:

$$n = \frac{K_{fs} V_f V_s C_o}{K_{fs} V_f + V_s} \qquad \textbf{Equation 1}$$

Where n is the amount extracted by the coating; $K_{fs}$ in the distribution coefficient between the fibre coating and the sample matrix; $V_f$ is the volume of the fibre coating; $V_s$ is the volume of the sample; and $C_o$ is the initial concentration of analyte in the sample (Lord and Pawliszyn, 2000).

In non-equilibrium conditions, the amount of extracted analyte is dependent on the sampling time, and this situation can be expressed with an exponential term showing the time dependence:

$$n = D_g A / Z \int C_{(t)} dt \qquad \textbf{Equation 2}$$

So within a short time interval, dt, the amount of analyte extracted is proportional to the integral of a sample concentration over time; the diffusion coefficient of analytes in gaseous phase, $D_g$; area of the needle opening, A; and inversely proportional to the distance of the coating from the needle opening, Z (Pawliszyn, 1999).

As can be seen from equation 2, there is a direct proportionality between the amount of extracted analyte and its initial concentration in the sample, so, once SPME conditions and sampling time are held constant, quantification by SPME is possible even before reaching equilibrium (Martínez-Uruñuela et al., 2005).

However, there is a high risk of quantitative errors linked to the use of a microextraction technique in complex media such as wines. So, for quantification purposes, and to remediate the difference in sensitivity of SPME fibres towards different volatiles, there is a need for standard calibration curves to evaluate the headspace concentration of the volatiles from GC peak area responses (Bhattacharjee and Panigrahi, 2007).

Figure 2 illustrates a commercial SPME device, manufactured by Supelco, Inc. (Bellefonte, PA.): A piece of stainless steel tubing holds the fibre which is mounted in a special holder; a modifiable depth gauge in the housing controls in a repeatable manner how far the needle enters the sample container or the injector. This is important as the fibre can be easily broken if it hits an obstacle (Lord and Pawliszyn, 2000).



**2-1- Extraction mode**

SPME can be performed in three basic modes: direct extraction of analytes from the liquid phase, headspace extraction, and extraction with membrane protection in case of the presence of interferers (Pawliszyn, 1999).

HS-SPME protects the fibre from adverse effects due to non-volatile high molecular weight substances present in the sample matrix, and allows modifications to the sample matrix such as pH adjustments (Balasubramanian and Panigrahi, 2011). In the same way, the extraction with membrane protection is used for the analysis of less volatile compounds.

**2-2- Different type of fibres**

The performance of SPME is critically dependent on the selection of appropriate coatings. The SPME fibre coating is primarily responsible for the extraction of analytes. Because of the need to extract a wide range of analytes including volatile and non-volatile, polar and non-polar compounds, it is essential to have SPME fibre coatings that can extract this range of analytes (Shirey and Pawliszyn, 2012).

The type of phase chosen determines the polarity of the coating. Polarity can provide selectivity by enhancing the affinity of the coating for polar analytes compared to a non-polar fibre coating. Essentially, all the SPME fibres are bipolar to different levels because they extract both polar and non-polar analytes, but it is the overall properties of the coating that decides polarity (Shirey and Pawliszyn, 2012).

The sampling fibres can be used several times, hundreds of analyses in the case of headspace analysis and dozens of times in the case of immersion analysis (Vas and Vékey, 2004).

## 3- Volatile wine components

Wine volatile profiles are characterized by compounds of different chemical classes and properties, occurring in concentrations ranging from ng/L to mg/L. Their contribution to the overall wine flavor is different, depending on their odor thresholds and concentrations (Jeleń et al., 2011). They are significantly affected by four major sources : genetics, the processing of the grapes, the fermentation process, and the maturation and ageing (Perestrelo et al., 2009).

Bortnowska (2010) reported that most flavor compounds are at least moderately lipophilic, and that the lipophilicity increases with molecular weight within a homologous series.

The volatile organic compounds in wines are responsible for their so-called 'bouquet' on sniffing the headspace from a glass, and for the odor/aroma component (palate-aroma) of the overall flavor perceived on drinking (Bakker and Clarke, 2011).

Figure 3 shows a typical red wine matrix. Most wine matrix ingredients (the components that are present in the wine) are capable of impacting the level of aroma volatiles in the headspace to some degree by a variety of mechanisms that change their solubility or volatility (Hartmann, 2003).

So, in sum and based on the consulted literature, to study a wine, either for the determination of its origin, its authenticity or its probable defects, it is essential to study its volatile composition. In fact, volatile compounds are the most studied molecules for the characterization of wine.

**3-1- Partition coefficient**

The distribution of volatile compounds between the liquid and gas phases depends on the vapor-liquid equilibrium (VLE), which is defined by the gas-liquid partition coefficient, $k_i$. The absolute volatility ($K_i$), or the activity coefficient ($\gamma_i$), representing the deviation from ideality, is related to the latter coefficient by the equation:

$$K_i = \frac{y_i}{x_i} = \frac{\gamma_i P^0_{i(T)}}{P_T} \qquad \textbf{Equation 3}$$

Where $x_i$ and $y_i$ are molar fractions in the liquid and gas phases, respectively; $P_i^0{}_{(T)}$ is the vapor pressure of pure component *i* at a temperature T ($P_a$); and $P_T$ is the total pressure ($P_a$) (Pozo-Bayón and Reineccius, 2009).

Volatile organic compounds with high boiling points but low solubility in water (or wine) have, perhaps surprisingly, very high partition coefficients. In contrast, compounds with low boiling points and high water solubility have low values (Pozo-Bayón and Reineccius, 2009).

De Roos (2003) ascribed the release kinetics of odorants from the matrix to two main factors which are volatility (thermodynamic factor) and resistance to mass transfer from the matrix to the air (kinetic factor).

The pH of the wine also has a significant influence on the concentration of some classes of the aroma compounds in the headspace. At low pH, the ionization of organic acids is suppressed (Hartmann, 2003) thereby increasing their volatility.

The studies of Robinson et al. and Rodriguez-Bencomo et al. (Robinson et al., 2009, Rodríguez-Bencomo et al., 2011) showed that the matrix influence on the compound-specific partition coefficient affects the partitioning of aroma compounds into the headspace.

These observations lead to the conclusion that, for a good implementation of SPME in wine so as to have the best conditions for extraction, target analytes should be properly identified, as should interactions between different wine components. With any complex matrix such as wine and where there are a wide range of analytes such as aroma compounds, a compromise solution should always be taken into consideration.

## 4- Application of headspace solid-phase microextraction in wine

The headspace method, in conjunction with GC–MS analysis, provides a high recovery, good linearity to analyze wine volatiles, over a wide range of concentrations and with a high sensitivity (Câmara et al., 2006).

### 4-1- Optimization

A properly optimized design procedure should result in good accuracy and precision together with low detection limits.

In wine aroma analysis, a number of articles related to the use of SPME in wine and its optimization were reviewed. It was found that every study takes into account parameters that the author considers as potentially influential. On average, four parameters are studied, usually extraction time; extraction temperature; salt addition; agitation and fibre type. Some authors fixed one parameter at a time and varied the others (Antalick et al., 2010) while a small number applied experimental designs (Rebière et al., 2010, Carrillo et al., 2006, Prouteau et al., 2004), which allowed the simultaneous variation of all the variables selected and the detection of potential interactions among them which would

not be found using classical one variable-at-a-time optimization (Noguerol-Pato et al., 2009). The fibre type used depends on the analytes to be extracted, in particular their polarity. As for extraction time and temperature, the best compromise between these factors and analyte response should be chosen, and extraction profiles established.

Among the parameters affecting the extraction quality, most are set to the same values in the various SPME methods published. This is the case with salt concentration for example, which is commonly sodium chloride at saturation, and also with magnetic stirring.

It is interesting to draw attention to the fact that classically, a univariate optimization approach is followed for HS-SPME without prior statistical determination of significant experimental factors. The improved understanding of the interactions between experimental conditions which can be achieved through multivariate statistical approaches can contribute to the development of sample/headspace equilibrium theory for headspace volatile analysis (Kalua and Boss, 2008).

Several experimental parameters need to be taken into account to optimize a new SPME method. The most important of these experimental parameters are discussed below.

**4-1-1- Fibre type**

Nearly all reviewed articles evaluated the impact of stationary phases on SPME optimization. Two general conclusions can be drawn from studies that optimized SPME as a function of fibre type:

1- The need to carefully match analyte and stationary phase polarities.

2- Sensitivity increases proportionally with the stationary phase thickness. On the other hand, very thin fibre coatings should be used whenever the sensitivity is sufficient because this will result in the shorter extraction times (Lord and Pawliszyn, 2000).

For specific applications, the choice of a suitable solid-phase depends on the class of compounds to be analyzed (Castro et al., 2008a).

Because it is thermostable and chemically neutral and can be desorbed at moderately high temperatures, PDMS fibre is often tried first for a new method, although its best efficiencies are observed for non-polar compounds (Spietelun et al., 2010), as it is a non polar material.

Nonetheless, in recent years the use of different phases based on the combination of different adsorbent/absorbent polymers, such as DVB/CAR/PDMS or CAR/PDMS, has been gaining popularity, since they can be used for the extraction of a broader range of analytes (Andujar-Ortiz et al., 2009), and have given the best results for the analysis of the totality of the aromatic fraction of wines (Castro et al., 2008a).

Spietelun et al. (2010) indicate that coatings combining polar and non-polar materials extract polar organic analytes from samples with a polar matrix composition with greater efficiency, while the simultaneous extraction of interferents is not possible. Furthermore,



 

carboxen gives PDMS/CAR coatings a greater specific area, as a result of which extraction of volatile organic compounds is highly effective.

Since the newly developed fibre DVB/CAR/PDMS finds applications over a broad range of analyte properties, it is the most-often used in the papers reviewed. With its three different phases, the extraction with DVB/CAR/PDMS occurs through three different mechanisms and so is particularly adapted for non-targeted extractions.

**4-1-2- Extraction time**

Extraction time is optimized in order to find the best time during which the equilibrium amount of compounds on the fiber is reached, thus the optimum time to hold the fiber in the headspace of the sample. Usually the sample is extracted for different predetermined exposure times. The optimal extraction time is determined depending on how fast the analytes pass to the headspace and are adsorbed by the fiber, and on their average peak areas.

Extraction time is optimized by determining the time required for an analyte to reach equilibrium between the sample matrix and the stationary phase. Generally, extraction yields increase over relatively long exposure times. For this reason, extraction times are set at a point where sensitivity and precision are maximized within a reasonable experimental time, and rarely at equilibrium (Krutz et al., 2003). Although, in principle, it is advisable to continue extraction until the system reaches equilibrium, in routine analyses there is often insufficient time to do so (Câmara et al., 2006).

A broad range of extraction times is presented in the literature with values ranging from 5 to 120 min, depending on the nature of analytes extracted, the temperature and the mode of extraction adopted.

In this way, the results in the study of Câmara (2006), showed that some compounds reached equilibrium after 30 to 40 min while others increase continuously over all the time considered (from 5 to 360 min) . Making a compromise between the duration of the analysis and the time of the extraction, an extraction time of 60 min for must and 120 min for wine samples was selected for subsequent analysis, because this provides sufficient extraction (>80%) of the analytes (Câmara et al., 2006).

In the reviewed articles, the most often used duration for extraction was 30 min, taking into consideration that the optimum extraction time is always a compromise between sensitivity, speed, precision and throughput (Risticevic et al.).

**4-1-3- Ionic strength**

Typically, analyte solubility decreases as ionic strength increases. A decrease in analyte solubility improves sensitivity by promoting analyte partitioning into the stationary phase. This “salting-out” effect is compound-specific.

In fact, most of the studied papers prefered to use salt addition at saturation (Risticevic et al.).

Nevertheless, Martínez-Uruñuela et al. (2004a) have established that the alteration of the ionic strength of the sample does not improve the extraction efficiency of 2,4,6-



 

trichloroanisole, 2,3,4,6-tetracholoranisole and pentachloroanisole from wines, the salting out effect depends on the type of compounds and optimization range tested.

Rebière et al. (2010) decided that no treatments should be applied to the wine sample to keep it as close as possible to the original matrix. This decision was a consequence of the experimental investigations that showed that although salting out did increase the signal intensity of different analytes; such an addition was not required as the sensitivity was already sufficient for their studied range of volatile compounds.

Nevertheless, rest of the literature shows clearly that the use of salt improves the extraction of analytes from wine.

**4-1-4- Extraction temperature**

Equilibrium time and analyte partitioning into the stationary phase vary inversely with the extraction temperature. Consequently, SPME methods can be optimized by selecting extraction temperatures where sufficient sensitivity is achieved within an acceptable time. The final temperature is chosen as a compromise between the need for sufficient sensitivity to detect target analytes at low concentrations, and to limit oversaturation of the fibre with the extraction of compounds at high concentrations (Rebière et al., 2010).

In fact, at room temperature, only volatile analytes are transported through the headspace; and the equilibration times for volatiles are therefore shorter in the headspace SPME mode than for direct extraction, under similar agitation conditions (Pawliszyn, 2012).

Based on the literature, the most reliable temperatures are between 30 and 45°C, just above room temperature and not too high so as to avoid modifying the original composition of the wine matrix.

**4-1-5- Stirring**

Stirring and sonication enhance analyte transfer from the matrix to the stationary phase, thus reducing the extraction time. Agitation facilitates the release of volatile compounds by increasing the surface of the liquid–vapor interface (Caldeira et al., 2007). For this reason, the equilibration time is inversely related to agitation rate. However, excessive agitation may in fact adversely affect equilibration time and precision, because it tends to be uncontrollable (Kataoka et al., 2000).

Although stirring the sample improves the extraction of only some of the volatile compounds, Andujar-Ortiz et al. (2009) decided to keep this condition, since the extraction of a greater amount of aroma compounds can be decisive in improving sensitivity when analyzing real wine samples. This choice was made in most of the reviewed papers as they showed that agitation during extraction is very critical.

**4-1-6- Sample volume**

Determining the volume of the sample is essential for the application of HS-SPME. The amount of analyte absorbed into the stationary phase increases as sample volume increases, and thus sensitivity increases. Few studies report optimizing SPME by adjusting sample volume. This fact is illustrated by equation 2, where the number of

moles of analyte extracted is proportional to the sample volume, thus indicating that increasing the sample volume should enhance the method sensitivity.

In their bulletin 923, Supelco (1998) explained that increasing the sample volume from 200 μL to 3 mL, while keeping the ratio of liquid to headspace constant (1:1), increases analyte adsorption by headspace SPME.

However, in their study on the determination of haloanisoles in wines and other alcoholic beverages using HS-SPME and GC-AED, Campillo et al. (2008) found that sensitivity decreases for all the analytes as the sample volume increases, probably because of the ethanol concentration and the presence of other compounds, that prevent the studied analytes from being adsorbed on the saturated fibre.

In his review, Kataoka et al (2000) explain that the critical point is that the vial size and sample volume should be the same during analysis by SPME. Similarly, Vinholes (2009) , in his study used the same vials from the same producer to perform the HS-SPME so as to avoid errors, since the precision of the results in a three-phase system (liquid, headspace and coating fibre) can be influenced by the headspace volume.

**4-1-7- Desorption conditions**

The most common procedure for desorbing analytes from the fibre in SPME is the thermal desorption in the injector of a gas chromatograph, eliminating the use of organic solvents.

To ensure an efficient desorption, the carrier gas around the fibre coating should have a high linear flow rate, during the thermal desorption in the GC injector port. This could be done by using an injector insert with a reduced internal diameter, like the 0.7mm id injection liner available from Supelco (Risticevic et al.).

Moreover, the splitless mode is more practical and should be used to increase the sensitivity (Pawliszyn, 1999). Almost all the reviewed papers used the splitless mode with a flow rate of the carrier gas of at least 1ml/min.

Desorption time should be as short as possible and carryover effects must be excluded. Thus, the highest temperature without damage of the coating used and the smallest diameter of the injector insert should be selected. The required desorption temperature may be close to the temperature of tolerance of the fibre.

Generally, the optimal desorption temperature is approximately equal to the boiling point of the least volatile analyte (Kataoka et al., 2000).

**4-1-8- Derivatization**

Some analytes are difficult to determine by SPME. Derivatization could remedy this problem in the case of unstable low molecular weight volatile compounds in a complex matrix like wine, by forming more stable derivatives. This can also add selectivity to the extraction method (Rodríguez-Bencomo et al., 2009).

Pawliszyn (1999) states that derivatization should only be considered in complex matrices and when necessary as it complicates the SPME procedure. Moreover selective



 

reactions producing specific analogues should be used in complex matrices during quantitation, because this approach results in less interference.

This step was considered essential for several studies such as for the detection of phenols (Martínez-Uruñuela et al., 2004b, Pizarro et al., 2007c, Viñas et al., 2009) or volatile thiols (Rodríguez-Bencomo et al., 2009) where they are transformed into less polar compounds thus improving their chromatographic analysis.

The derivatization process can be performed in several ways with SPME: (1) pre-extraction derivatization; (2) post-extraction derivatization; and (3) simultaneous extraction and derivatization. The choice of the derivatization strategy should be made based on the analytes of interest, the derivatization reagents and the type of sample matrix studied (Risticevic et al.).In wine, several compounds were studied after derivatization especially volatile carbonyls , such as aldehydes, due to the reactivity of the carbonyl group and the low specificity of their mass spectra (Zapata et al., 2010). Chlorophenols and thiols are also derivatized prior to their determination by GC, due to their high polarity (Martínez-Uruñuela et al., 2004b, Rodríguez-Bencomo et al., 2009).



**4-2- Applications**

The number of publications on the application of HS-SPME in the analysis of wine volatiles has increased regularly over the last decade. The most frequently encountered compounds in the reviewed literature are listed with their characteristics in table 2.

Numerous methods have been developed to analyze and identify wine volatiles using the HS-SPME/GC-MS approach (Table 3). A survey of these methods is presented in more

detail to give a comprehensive, global vision about the practice of HS-SPME and also to compare different approaches.

**4-2-1- Study of off-flavors in wine**

Some volatiles that are usually present in wine aroma and contribute positively to it, if present at high concentrations may cause wine off-flavors that negatively affect wine quality. So it is necessary for winemakers to control the concentrations of these compounds in their wines. Thus, we review some studies that examine the application of HS-SPME to detect these molecules below their sensory threshold.

The most studied off-flavors in wine, due to their high impact on wine aroma perception (relatively low sensory threshold), are: 3-alkyl-methoxypyrazine, trichloroanisole, volatile sulfur compounds and volatile phenols.

- **3-alkyl-2-methoxypyrazine**

Alkylmethoxypyrazines are found in green bell peppers and also in Cabernet Sauvignon and Sauvignon Blanc grapes. They are key varietal aroma characteristic of these wines. Excessive levels of these compounds easily overwhelm and unbalance the wine. The most important 3-alkyl-2-methoxypyrazine in grapes and wines is 2-methoxy-3-isobutyl (MIBP), and its optimum concentration is in the low range of 8-15ng/l. Concentrations higher than 30ng/l are associated with unbalanced aromas (Godelmann et al., 2008).

In the study by Galvan et al. (Galvan et al., 2008), the CAR/PDMS fibre showed better linearity and less variability for the 3-alkyl-2-methoxypyrazine than the

DVB/CAR/PDMS. In fact, DVB/CAR fibres gave high recoveries and better chromatograms of 3-alkyl-2-methoxypyrazine, when used with the NPD detector and Agilent DB Wax column. 12 mL of wine were placed in 20 mL headspace vial with 3.6 g NaCl and a stirring bar. As for Godelmann et al. (2008), the best results for the determination of 3-alkyl-2-methoxypyrazines in wine were obtained with CAR/PDMS, 5 mL wine in 20 mL headspace vial and 2 g NaCl. It is worth noting that the former study indicated the RSD below 20%, while the validation method in the latter study gave the detection and quantification limits without mentioning the relative standard deviations of replicates.

The changes in 3-alkyl-2-methoxypyrazine concentrations during bottle aging were studied in relation with closure/packaging options by Blake et al. (2009). 3-Alkyl-2-methoxypyrazines were quantified in wines at bottling and after 3, 6, 12, and 18 months, using HS-SPME, with PDMS/DVB/CAR, for 30 minutes at 40 $^{o}$C. Average RSDs from duplicate measurements across all wine samples for all volatile compounds were below 8%, which was good compared to other studies.

- **Trichloroanisole**

The presence of 2,4,6-trichloroanisole (TCA) in cork stopper is the main cause of the cork taint, a well-known off-flavor problem in wine. (Özhan et al., 2009).

HS-SPME coupled to GC is currently the most commonly employed technique for the extraction, pre-concentration and separation of compounds responsible of cork taint in wine (Bianco et al., 2009).

Campillo et al. (2008), selected the 50/30 DVB/CAR/PDMS fibre with an extraction time of 60 minutes, ionic strength of 20 %, and extraction temperature of 75$^{o}$C. The headspace extraction mode was selected in order to increase reproducibility and extend fibre life-time. Afterward, 3 mL of wine and 4mL of water were placed in 15 mL vials, to perform the analysis. This study explained in detail all the steps adopted for the optimization of the HS-SPME leading to a standard deviation below 7% for 5 replicates. The optimised extraction time was slightly higher than in other studies for headspace analysis.

Vlachos et al. (2007) however, used the PDMS fibre for the headspace extraction of 10 mL wine in 20 mL headspace vial. The extraction was carried out at 25 $^{o}$C, for 30 min. In order to overcome matrix effects, the standard addition calibration technique was applied. The detector used was the ECD. The same fibre was used by Bianco et al. (2009) after comparison with the CAR/DVB/PDMS fibre. PDMS phase maintained good durability and showed good precision with the investigated off-flavor compounds. So 3 mL of wine in 5 mL vial where extracted at 40 $^{o}$C for 30 min. This latter method gave an excellent repeatability of 3.24 %, based on 6 replicates.

HS-SPME procedures with GC-ECD detection were successfully applied by Özhan et al. (2009) for the detection of chloroanisoles and chlorophenols in wines and cork samples. A headspace sampling was conducted using 50 mL vials, containing 20 mL of liquid sample; the extraction was performed at 25 $^{o}$C for 30 min.

Since the methylation of phenols is the main source of anisoles in wine (Özhan et al., 2009), it can be understood that the chlorophenols are the direct precursors of

chloroanisoles. Therefore several studies involve the determination of both chloroanisoles and chlorophenols in wine (Özhan et al., 2009, Martínez-Uruñuela et al., 2005, Pizarro et al., 2008, Pizarro et al., 2007c).

The aforementioned articles indicate that more effort should be made to include derivatization processes into the determination of the chloroanisoles and halophenols responsible for cork-taint.

- **Sulfur compounds**

Volatile sulfur compounds usually play a pivotal role in the aroma of foodstuffs and beverages, even at low concentrations, often with typifying scents but also with off-flavors (Fedrizzi et al., 2007). Their characteristic odors range from rotten eggs to cooked cabbage and are associated with the reduction off-flavors found in some wines (López et al., 2007).

Usually, the "heavy" off-flavor compounds (boiling point >90 $^{o}$C) in wine were analyzed by liquid –liquid extraction. In the work of Fedrizzi et al.(2007), 13 sulfur volatiles (boiling points from 35 $^{o}$C to 231 $^{o}$C) were quantified in wine by HS-SPME/GC-MS using a CAR/DVB/PDMS 50/30 fibre; their relative standard deviation of 5 replicates was below 10% for the most of the compounds studied.

This is also true for López et al. (2007) who suggested a new procedure for the detection of sulfur in wine: in a sealed vial containing 4.9 mL of saturated NaCl brine, the air is entirely displaced with nitrogen, the wine (0.1 mL) and the internal standards (0.02 mL) are then introduced with a syringe through the vial septum. This sample is extracted at 35

$^{o}$C for 20 min. The sample dilution was the most obvious approach for them to minimize the influence of the matrix. This method seems to be suitable for sulphur analysis knowing that sulphur compounds are very sensitive to oxidation and degradation.

Precautions should therefore always be taken for the analysis of volatile sulphur compounds. Standards should always be used and a correct quantitative evaluation of these standards should be made often, the detector should preferably be a sulphur-specific (López et al., 2007).

Another way to avoid sulphur analysis problems is derivatization, which was used by Capone et al. (2011), with a PDMS/DVB fibre, at 80 $^{o}$C with agitation and salt addition.

It is worth mentioning that, to date, not all problems in the analysis of volatile sulphur compounds in wines have been solved, and more efforts need to be made, especially in quantification of these compounds.

- **Volatile phenols**

Wine spoilage caused by the yeast *Brettanomyces bruxellensis*, sometimes referred to as "Brett character", results in the production of several volatile compounds and a broad spectrum of flavours and aromas. Ethyl phenols are the best-known markers of this defect (Romano et al., 2009).

Castro Mejías et al. (2003) applied HS-SPME for the analysis of volatile phenols in wine. After 5 min at 60 $^{o}$C, the CW– DVB fibre was exposed to the headspace of the sample for

50 min, with stirring and salt addition. The method was successfully applied with recoveries close to 100%, and repeatability values lower than 16%.

On the other hand, Pizarro et al. (2007a) demonstrated the existence of a matrix effect in the analysis of compounds responsible for Brett character in wine, when an HS-SPME method is used with a carbowax/divinylbenzene (CW/DVB) fibre. For this reason, they proposed multiple headspace solid-phase microextraction as an alternative strategy to single HS-SPME.

Multiple-headspace solid-phase microextraction (MHS-SPME) has been also developed for the determination of phenols in wine (Pizarro et al., 2007b). It is an important procedure to avoid complex matrix effects in wine. Because of its repeatability and reproducibility, it is gaining in popularity.

### 4-2-2 Study of aroma compounds in wine

Given that HS-SPME is particularly appropriate for application in the field of volatile compounds, the characterization of different wines has been conducted using this method. In fact, due to the specificity of food matrices, SPME is used almost exclusively as headspace extraction method, and when looking into the types of food in which SPME was used for the analysis of flavor/volatile compounds, the largest group was wine (Jeleń et al., 2012).

The HS-SPME/GC-MS method is suitable for identifying and quantifying oak volatile compounds at trace levels in wine. The study by Carrillo et al showed that high

temperature and the addition of sodium chloride to the samples improved the extraction process, with an extraction time of 60 min and agitation in order to shorten the equilibrium time of certain compounds (Carrillo et al., 2006). This was a good procedure when used with either internal standard or standard addition calibration.

The dynamic HS-SPME/GC–MS method was found to be well-suited for the analysis of free terpenes and $C_{13}$ norisoprenoids in wine, due to its selectivity and sensitivity. An optimized methodology was developed, based on 85 µm PA fibre, headspace sampling mode and an extraction time of 60 min for musts, and 120 min for wines at 40 $^{o}$C (Câmara et al., 2006). The results show that Boal, Malvazia, Sercial and Verdelho varieties have different profiles of terpenoid compounds. Moreover, Vinholes et al. (2009), pointed out that the SPME method allowed them to determine $C_{13}$ norisoprenoids in the headspace of white and red wines in only 13 min (10 min SPME plus 3 min instrumental analysis), instead of using more time-consuming standard methods.

HS-SPME allowed Bosch-Fusté et al. (2007) to obtain the most important volatile compounds of each chemical family in wine. In the same way, Tao et al. (2008) identified some sixty nine volatile compounds of young red wines from *Vitis vinifera* cv. Cabernet Sauvignon in Changli County (China).

A mixed level factorial design was used to determine the appropriate extraction conditions for the determination of primary and secondary aroma compounds in Mencia red wine by HS-SPME. The proposed optimal analytical procedure was: 30 mL of sample with the addition of 30 % of NaCl, a DVB/CAR/PDMS fibre, 25 $^{o}$C extraction

temperature, 45 min extraction time with agitation, 250 $^{o}$C desorption temperature and 10min desorption time (Noguerol-Pato et al., 2009).

The effect of yeast on volatile wine aroma compounds was studied by Comuzzo et al. (2006) with a 2 cm 50/30 DVB/CAR/PDMS fibre. SPME was run at 12 and 37 $^{o}$C, for 15 min; these temperatures were chosen to simulate either the serving temperature of white wines (12 $^{o}$C) or mouth temperature during tasting (37 $^{o}$C). Simulation in this case is very useful and is facilitated by the use of SPME technique.

Zhang et al. (2010), while studying the classification of Chinese red wines from different varieties, found that ageing effect is responsible for most of the variation in the volatile composition of the analyzed wine.

Finally, the HS-SPME method was used successfully to monitor aromatic compounds during alcoholic fermentation of Syrah, in order to control the oenological process; 5mL of wine were placed in 15 mL vial and extracted at 40 $^{o}$C for 30 min (Zhang et al., 2011).

Depending on the fibre used, the operating conditions of the SPME are different in the articles reviewed. This is due to the broad spectrum of compounds that can be extracted from a wine matrix. For this reason, there is a need for new SPME fibre operating conditions in order to further broaden the range of extracted analytes.

### 4-3 Problems encountered using SPME

The limitations of SPME include analyte carry-over, fibre damage at extreme pH, and salt-related problems. Furthermore, SPME sensitivity is limited in complex matrices.

This is the case in wine analysis where the matrix is the main factor affecting SPME performance, due to its complexity and the low taste and odor threshold levels of the aroma compounds of interest (Gómez-Ariza et al., 2006).

To avoid analyte carryover, it is possible to extend desorption time or run a blank between two extractions to be sure that all the analytes have been desorbed. As for fibre damage, it is recommended to use headspace analysis to extend the fibre's life expectancy.

Zapata et al. (2010) suggest that SPME method suffers important matrix effect. However, multiple-headspace solid-phae microextraction (MHS-SPME) is a modification of conventional HS-SPME that can help to reduce these effects.

Another important drawback for the analysis of wine by HS-SPME is the effect of ethanol (the most abundant volatile compound in wine) on the fibre which can be the cause of low repeatability. However, this problem can be minimized by using an adequate internal standard (Carrillo et al., 2006).

Optimization of operating conditions is always required as there is a bias toward higher recoveries of lower boiling, less polar compounds. So It is important to focus on the analytes of interest when optimizing sampling conditions, and to remain aware that optimum conditions for one set of compounds will not necessarily be optimal for another set of compounds (Castro et al., 2008b).



 

Moreover, the factors affecting precision should be taken into consideration. Thus, the operating parameters such as time, temperature and agitation should be kept constant for all samples and standards to be analysed, the same type of vial should be used for all samples, the fibre should be replaced regularly, the injector septum should be replaced frequently to avoid the coring inside the liner, and the fibre sampling depth in the vial should be kept constant in all samples.

As for the reproducibility, SPME can be fully automated, which improves the accuracy of the results.



### 4-4 The use of HS-SPME in wine literature

It is impossible to gather and comment all HS-SPME applications in one document. Thus, in order to better review and evaluate the conditions used for HS-SPME in wine analysis, several studies between 2006 and early 2012 were compiled, according to relevance and the optimization of the method The conditions of use and the SPME parameters considered in each article are summarized in table 3 where the articles are presented in chronological order.

This table highlights the principal operating conditions used in several studies. It is to be noted that the repeatability was not very good in all studies, especially where the compounds were chemically unstable. This being so, advantage should be taken of the information in these studies without necessarily adopting exactly the same conditions, but rather as starting points for further optimization studies to attain adequate repeatability

and precision levels, even though new articles adopted already optimized procedure of HS-SPME.

## 5- Conclusion

Sample preparation considerably influences the reliable and accurate analysis of complex matrices such as food samples. The HS-SPME technique described in this review is remarkably effective as a sample preparation procedure for qualitative and quantitative analyses. As extraction and concentration are combined, all of the extracted analytes are introduced into the analytical system (Kataoka et al., 2000).

From this literature review on HS-SPME applied to wine, we can distil general ideas about the way to solve problems concerning the use of HS-SPME in wine aroma analysis.

HS-SPME is sufficiently sensitive to meet legislative requirements related to low detection and quantification limits, as well as method accuracy and precision, but in order to achieve the best performance, the protocol should be adjusted to each case. An optimization procedure should therefore be conducted before working with HS-SPME. To do this, several preliminary experiments should be run, in order to determine the most suitable conditions for microextraction.

The common operating conditions adopted by most of the authors were: the 3 phase DVB/CAR/PDMS fibre, stirring condition, moderate extraction temperature (between 35 and 45 $^{o}$C), and volume ratio of sample/vial of ½.

Most of the studies were concerned with geographical or botanical authenticity of the wine. Another important group was concerned with detecting particular contaminations or wine defects. A small number studied the influence of chemical or technological processes, the analysis of selected compounds or determining volatile profiles.

The use of HS-SPME for food analysis in general and wine analysis in particular, is expected to find increasing applications and better recognition as improved techniques and newer fibre technologies emerge.



 

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**Table 1:** Types of fiber coating available and their extraction mechanism and polarity (Shirey and Pawliszyn, 2012).

| **Type of Coating*** | **Extraction Mechanism** | **Polarity*** |
|---|---|---|
| 7 µm PDMS | Absorbent | Non-polar |
| 30 µm PDMS | Absorbent | Non-polar |
| 100 µm PDMS | Absorbent | Non-polar |
| 85 µm PA | Absorbent | Polar |
| 60 µm PEG | Absorbent | Polar |
| 15 µm Carbopack Z-PDMS | Absorbent | Bipolar |
| 65 µm PDMS-DVB | Adsorbent | Bipolar |
| 55 µm/30 µm DVB/Carboxen-PDMS | Adsorbent | Bipolar |
| 85 µm Carboxen-PDMS | Adsorbent | Bipolar |

*The nature of the different layers of the fibre: PDMS: Polydimethylsiloxane, DVB: divinylbenzene, PA: Polyacrylate, PEG: Polyethylglycol

*Polarity : P= Polar; N= Non-polar; B= Bipolar

**Table 2:** Characterization of main wine volatiles encountered in literature.

| wine components | Odor threshold (mg/L) | LogP |
|---|---|---|
| **Alcohols** | | |
| 1-propanol | 306[a] | 0.25[e] |
| 2-Methyl-1-propanol | 75[a] | 0.76[f] |
| 3-Methyl-1-butanol | 60[a] | 1.22[e] |
| Cis-3-hexenol | 1[a] | 1.61[g] |
| 1-Hexanol | 1.1[a] | 2.03[f] |
| 2-Phenethyl alcohol | 200[a] | 1.36[f] |
| **Acetates** | | |
| Isoamyl acetate | 0.16[a] | 2.26[e] |
| Hexyl acetate | 0.67[a] | 2.83[g] |
| 2-Phenethyl acetate | 1.8[a] | 2.3[g] |
| **Esters** | | |
| Ethyl hexanoate | 0.08[a] | 2.83[f] |
| Ethyl octanoate | 0.58[a] | 3.9[e] |
| Ethyl propanoate | 1.8[a] | 1.21[e] |
| Ethyl decanoate | 0.5[a] | 4.79[f] |
| Ethyl acetate | 12[a] | 0.71[e] |
| Ethyl butyrate | 0.4[a] | 1.85[e] |

| | | |
|---|---|---|
| Diethyl succinate | 500[b] | 1.2[f] |
| **Volatile fatty acids** | | |
| Decanoic acid | 6[a] | 4.09[e] |
| Hexanoic acid | 3[a] | 1.72[e] |
| Octanoic acid | 10[a] | 2.74[e] |
| **Monoterpernic alcohols** | | |
| Nerol | – | 3.56[g] |
| Linalool | 0.015[a] | 2.97[f] |
| **Aldehydes** | | |
| nonanal | 0.001[b] | 3.46[e] |
| Decanal | 1[b] | 4.09[e] |
| **Others** | | |
| Ionone | 0.005[a] | 3.84[g] |
| 3-methyl-2-methoxypyrazine | 0.004[c] | 0.32[e] |
| 2,4,6-trichloroanisole | 0.00001[d] | – |

*Log P<1: Polar - Log P=1-3: Mid-Polar - Log P>3: Non-Polar

a: (Peinado et al., 2004)

b: (Jiang et al., 2013)

c: (Alberts et al., 2013)

d: (Mazzoleni and Maggi, 2007)

e: (Thomsen et al., 2014)

f: (Mitropoulou et al., 2011)

g: (Rodríguez-Bencomo et al., 2011)



 

**Table 3**: Application of HS-SPME/GC-MS to the study of wine volatiles.

| Year | Ref | Analyte studied | Fibre type | Salt addition | Sample volume | Vial volume | Stirring | Extraction temperature °C | Equilibrium time | Extraction time |
|---|---|---|---|---|---|---|---|---|---|---|
| **2012** | **(Sagratini et al., 2012)** | Aroma profile[1] | PDMS | 20% | 13ml | 20ml | Yes | 35 | 13min | 30min |
| **2012** | **(Welke et al., 2012)** | Volatile profile[2] | DVB/CAR/PDMS | 10% | 10ml | 30ml | Yes | 40 | No | 60min |
| **2012** | **(Jeleń et al., 2012)** | Volatile profile | _ | _ | 2ml | 10ml | Yes | 35 | 30min | 30min |
| **2011** | **(Zhang et al., 2011)** | Volatile profile | DVB/CAR/PDMS | 30% | 1ml | 20ml | No | 45 | 10min | 45min |
| **2011** | **(Dall'Asta et al.,** | Volatile profile | PDMS, 100 | 12,50 % | 8ml | 15ml | Yes | 40 | 10min | 30min |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **2011)** | | | | | | | | | |
| **2011** | **(Xi et al., 2011)** | Volatile profile | CAR/PDMS | 50% | 10ml | 20ml | Yes | 23 | _ | 10min |
| **2011** | **(Weldegergis et al., 2011)** | Volatile profile | DVB/CAR/PDMS | 20% | 5ml | 15ml | Yes | 40 | 30min | 30min |
| **2011** | **(Perestrelo et al., 2011)** | Furans, Lactones, Volatile Phenols | DVB/CAR/PDMS | 0,5 | 1ml | 5ml | yes | 60 | 5min | 20min |
| **2010** | **(Antalick et al., 2010)** | Esters | PDMS | 35% | 10ml | 20ml | Yes | 40 | 2min | 30min |
| **2010** | **(Tao and Zhang, 2010)** | Aroma profile | PDMS | 20% | 10ml | 15ml | Yes | 40 | 10min | 30min |
| **2010** | **(Rebière et al.,** | Volatile profile | DVB/CAR/PDMS | _ | 10ml | 20ml | No | 40 | No | 20min |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **2010)** | | | | | | | | | |
| **2010** | **(Jiang and Zhang, 2010)** | Aroma profile | PDMS | 25% | 8ml | 20ml | No | 40 | No | 30min |
| **2010** | **(Zhang et al., 2010)** | Volatile profile | PDMS | | 10ml | 20ml | No | 45 | No | 20min |
| **2009** | **(D'Auria et al., 2009)** | Aroma profile | DVB/CAR/PDMS | 20% | 5ml | 15ml | Yes | 40 | No | 30min |
| **2009** | **(Andujar-Ortiz et al., 2009)** | Volatile profile | CAR/PDMS | _ | 8ml | 20ml | Yes | 40 | 10min | 20min |
| **2009** | **(Noguerol-Pato et al., 2009)** | Main odorants | DVB/CAR/PDMS | 30% | 30ml | 40ml | Yes | 25 | 10-15min | 45min |
| **2009** | **(Bianco et al.,** | Trichloroanisoles | PDMS | 20% | 3ml | 5ml | Yes | 40 | No | 30min |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **2009)** | | | | | | | | | |
| **2009** | **(Vinholes et al., 2009)** | Norisoprenoids | CW-DVB | 10% | 20ml | 60ml | Yes | 40 | No | 10min |
| **2009** | **(Perestrelo et al., 2009)** | Higher alcohol acetates, isoamyl esters, ethyl esters | DVB/CAR/PDMS | 10% | 10ml | 20ml | Yes | 25 | No | 60min |
| **2009** | **(Goldner et al., 2009)** | Volatile thiols | DVB/CAR/PDMS | | _ | _ | No | 110 | No | 30min |
| **2009** | **(Rodríguez-Bencomo et al., 2009)** | Ethyl carbamate | CW-DVB | 12,5% | 5ml | 15ml | Yes | 71 | 60min | 60min |
| **2009** | **(Blake et al., 2009)** | 3-Alkyl-2-methoxypyrazine | DVB/CAR/PDMS | 0,3 | 10ml | _ | yes | 40 | No | 30 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **2008** | **(Campillo et al., 2008)** | Haloanisoles | DVB/CAR/PDMS | 20% | 7ml | 15ml | Yes | 75 | No | 60min |
| **2008** | **(Kotseridis et al., 2008)** | 3-alkyl-e-methoxypyrasine | DVB/CAR/PDMS | 30% | 10ml | 20ml | Yes | 37 | No | 30min |
| **2008** | **(Pizarro et al., 2008)** | Chloroanisoles and chlorophenols | DVB/CAR/PDMS | _ | 4ml | 20ml | Yes | 70 | 15min | 60min |
| **2008** | **(Zhang and Zhang, 2008)** | Off-flavours | DVB/CAR/PDMS | 30% | 5ml +water | 20ml | Yes | 45 | 3min | 60min |
| **2008** | **(Tao et al., 2008)** | Volatile profile | DVB/CAR/PDMS | _ | 25ml | 50ml | No | 37 | 15min | 15min |
| **2007** | **(Bosch-Fusté et al.,** | Volatile profile | PDMS | 12,5 % | 8ml | 15ml | Yes | 45 | No | 15min |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **2007)** | | | | | | | | | |
| **2007** | **(Vlachos et al., 2007)** | 2,4,6-Trichloroanisoles | PDMS | 30% | 10ml | 20ml | Yes | 25 | No | 30min |
| **2007** | **(Câmara et al., 2007)** | Terpenoids | PA | 12,50 % | 2.4ml | 4ml | Yes | 40 | No | 120min |
| **2007** | **(López et al., 2007)** | Volatile sulfurs | CAR/PDMS | 7% | 5ml | 20ml | Yes | 35 | 5min | 20min |
| **2007** | **(Fedrizzi et al., 2007)** | Volatile sulfurs | DVB/CAR/PDMS | 25% | 20ml | 30ml | Yes | 35 | 5min | 30min |
| **2007** | **(Boutou and Chatonnet, 2007)** | Volatile profile | DVB/CAR/PDMS | _ | 5ml | 10ml | Yes | 35 | No | _ |
| **2007** | **(Câmara et al., 2007)** | Terpenoids | PA | 30% | 2.4ml | 4ml | Yes | 40 | No | 120min |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **2006** | **(Comuzzo et al., 2006)** | Volatile profile | PDMS | 30% | 5ml | 15ml | Yes | 25 | No | 30min |
| **2006** | **(Carrillo et al., 2006)** | Volatile oak compounds | _ | 30% | 10ml | 20ml | No | 70 | 10min | 60min |

[1] Aroma profile: a profile containing compounds known to contribute positively to wine bouquet. Sometimes, sensory analysis is conducted for this purpose.

[2] Volatile profile: general profile of compounds in the wine with no special attribute or sensory analysis.

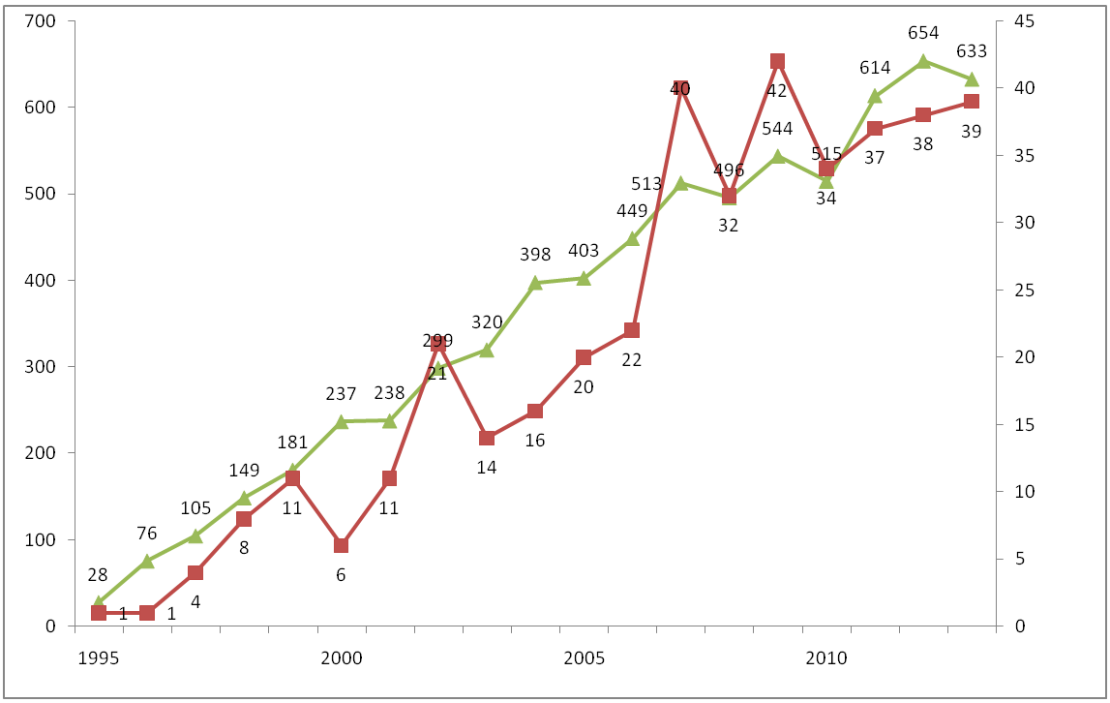


**Figure 1:** Number of published articles between 1998 and 2013 related to HS-SPME (▲) and HS-SPME/Wine (■) aroma applications.

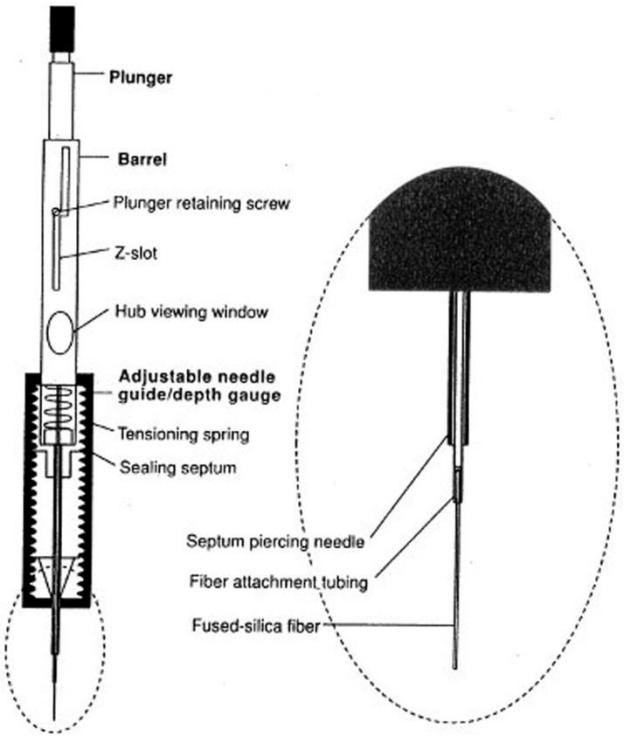


**Figure 2**: Design of the commercial SPME device (Supelco, Inc), reproduced from (Kataoka et al., 2000).

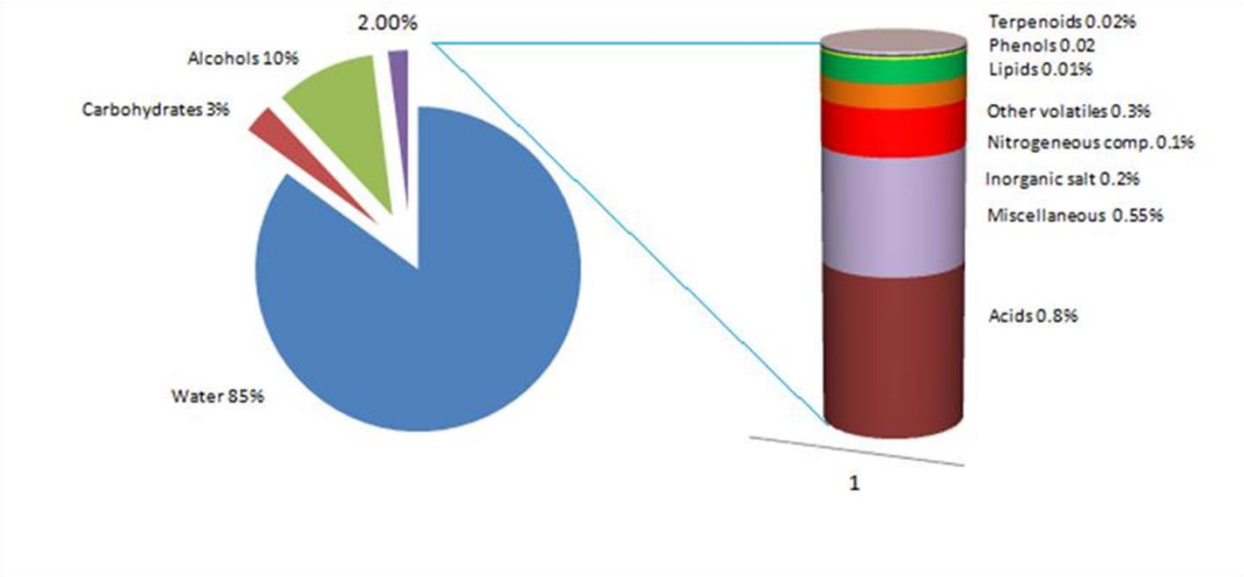


**Figure 3:** Typical wine composition (Hartmann, 2003).