Application of Gas Chromatography on the Evaluation of Grape and Wine Aroma in Atlantic Viticulture (NW Iberian Peninsula)

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1. Introduction

The volatile fraction of wine determines to a great extent its aroma, which is one of the most important characteristics influencing wine quality and consumer preferences. The volatile compounds are able to stimulate the sensorial organs that are responsible for the olfaction. These compounds correspond to small molecules, of medium hydrophobicity and molecular weight generally, between 30 g/mol and 300 g/mol (Morrot & Brochet, 2000).

Therefore, flavour is the sensation perceived by the brain when the olfactory epithelium is punched by a fraction of molecules, which were vaporized in the glass – orthonasal route – or put in contact with the mouth – retronasal route (Portmann, 2000). Consequently, the intensity of the olfactory sensation is not simply dependent on the concentration of the volatile compound in the liquid phase, but depends also on its volatility, its vapour pressure and its perception threshold (Meilgaard et al., 1999).

The olfactory perception threshold could be defined as the minor stimulus which could be able to promote an olfactory sensation in at least 50 % of a jury of a sensory panel. If the tasters are able to identify the odour, a recognition threshold is specified. On the other hand, if the volatile compound is already present in the tasting solution, a difference threshold could be defined as the minor addition of the substance susceptible to promote a change in the sensory stimulus (Dubois, 1993; Meilgaard et al., 1999).

1.1 Classification and origin of volatile compounds

The huge complexity of wine aroma may be attributed to the higher number of volatile constituents which are the result of a longer biotechnological sequence and the broad variability of concentrations from few ng L^{-1} to hundreds of mg L^{-1}. Furthermore, each volatile compound presents its own olfactory perception threshold which is, in turn, influenced by the other constituents of the wine.

Depending on the origin, and considering the biotechnological sequence of winemaking, wine flavour can be classified into four different groups (Bayonove et al., 1998): varietal aroma, typical of grape variety, which depends essentially on soil, climate, phytotechny, sanitary conditions and degree of ripeness; pre-fermentative aroma, originated during grape processing and subsequent operations, namely transport, pressing, maceration, and clarification; fermentative aroma, produced by yeasts during alcoholic fermentation and lactic acid bacteria during malolactic fermentation, which depends mainly on fermentation temperature and microorganism species; post-fermentative aroma, which results from transformations occurred during conservation and ageing of wine.

More than 1000 volatile compounds could be found in wine (Poláskova et al., 2008), but only less than 10 % may contribute to the flavour. The participation of each compound varies considerably by qualitative reasons, positive or negative olfactory impact, or by quantitative reasons, linked to the perception threshold. Additionally, the antagonism or the synergism between compounds should be also taking in account. Table 1 shows some relevant volatile compounds contributing to wine aroma and the respective perception thresholds and descriptors. It must be noted that, for each compound, a broad range of perception thresholds could be found in literature. This could be explained by the purity of the compounds in test, the composition of the matrix used in the essays (water; hydroalcoholic solution and its ethanol content; characteristics of the model wine solution; type of wine) and the adopted tasting method, orthonasal or retronasal.

Family/Compound	Perception Threshold, PT [a] / (μg L^{-1})		Descriptor [b]	Reference
Monoterpenes				
Z-rose oxide	0.2	hydro-alcoholic solution; retronasal	green, floral	[a] Guth, 1997; [b] Ong & Acree, 1999
Nerol oxide	100	water	fragrant	[a,b] Simpson, 1979
Linalool	25.2	model wine solution; orthonasal	lemon	[a] Ferreira et al., 2000; [b] Escudero et al., 2004
HO-trienol	110	water	linden	[a] Simpson, 1979; [b] Ribéreau-Gayon et al., 2000
α-terpineol	250	model wine solution; orthonasal	pine	[a] Ferreira et al., 2000; [b] Meilgaard, 1975
Nerol	400	water	lime, roses	[a] Ribéreau-Gayon et al., 2000; [b] Meilgaard, 1975
Geraniol	36	model wine solution; orthonasal	rose-like, citrus-like	[a] Escudero et al., 2004; [b] Czerny et al., 2008
Wine lactone	0.01	hydro-alcoholic solution; retronasal	sweet, coconut	[a] Guth, 1997; [b] Guth, 1996

Family/Compound	Perception Threshold, PT [a] / (μg L^{-1})	Descriptor [b]	Reference
Methoxipyrazines			
3-isobutyl-2-methoxypyrazine	0.0039	water; orthonasal	bell pepper-like	[a,b] Czerny et al., 2008
3-isopropyl-2-methoxypyrazine	0.0062	water; orthonasal	earthy, pea-like	[a,b] Czerny et al., 2008
C$_{13}$-norisoprenoids			
β-damascenone	0.05	hydro-alcoholic solution; retronasal	sweet, apple	[a] Guth, 1997; [b] Escudero et al., 2004
β-ionone	0.09	model wine solution; orthonasal	flowery, violet-like	[a] Ferreira et al., 2000; [b] Czerny et al., 2008
Thiols			
4-mercapto-4-methyl-2-pentanone	0.0008	model wine solution; orthonasal	box tree, broom	[a,b] Tominaga et al., 1998a
4-mercapto-4-methyl-2-pentanol	0.0055	model wine solution; orthonasal	citrus zest	[a,b] Tominaga et al., 1998b
3-mercaptohexyl acetate	0.0042	model wine solution; orthonasal	box tree, passion fruit	[a] Tominaga et al., 1996; [b] Tominaga et al., 1998a
3-mercapto-1-hexanol	0.06	model wine solution; orthonasal	passion fruit, grapefruit	[a, b] Tominaga et al., 1998b
C$_6$-compounds			
Z-3-hexen-1-ol	400	hydro-alcoholic solution; retronasal	lettuce-like	[a] Guth, 1997; [b] Czerny et al., 2008
Alcohols			
2-methyl-1-butanol	1 200	water; orthonasal	alcohol, solvent	[a] Czerny et al., 2008; [b] Meilgaard, 1975
3-methyl-1-butanol	30 000	hydro-alcoholic solution; retronasal	alcohol, solvent	[a] Guth, 1997; [b] Meilgaard, 1975
2-phenylethanol	14 000	model wine solution; orthonasal	roses, perfumed	[a] Ferreira et al., 2000; [b] Escudero et al., 2004
Esters			
Ethyl butyrate	20	hydro-alcoholic solution; retronasal	fruity	[a] Guth, 1997; [b] Czerny et al., 2008
Ethyl hexanoate	14	model wine solution; orthonasal	fruity, apple	[a] Ferreira et al., 2000; [b] Meilgaard, 1975
Ethyl octanoate	5	model wine solution; orthonasal	fruity, fresh	[a] Ferreira et al., 2000; [b] Escudero et al.,2004
Ethyl decanoate	200	model wine solution; orthonasal	fruity, fatty acid	[a] Ferreira et al., 2000; [b] Meilgaard, 1975
Ethyl 2-methylbutyrate	18	model wine solution; orthonasal	fruity	[a] Ferreira et al., 2000; [b] Czerny et al., 2008

Family/Compound	Perception Threshold, PT [a] / ($\mu g \, L^{-1}$)		Descriptor [b]	Reference
Ethyl 3-methylbutyrate	3	model wine solution; orthonasal	fruity, blueberry-like	[a] Ferreira et al., 2000; [b] Czerny et al., 2008
3-methylbutyl acetate	30	hydro-alcoholic solution; retronasal	banana	[a] Guth, 1997; [b] Meilgaard, 1975
2-phenylethyl acetate	250	hydro-alcoholic solution; retronasal	flowery	[a] Guth, 1997; [b] Escudero et al., 2004
Fatty acids				
3-methylbutyric acid	33.4	model wine solution; orthonasal	fatty, rancid	[a] Ferreira et al., 2000; [b] Escudero et al., 2004
2-methylbutyric acid	3 300	hydro-alcoholic solution; retronasal	sweaty, cheesy	[a] Guth, 1997; [b] Czerny et al., 2008
Hexanoic acid	420	model wine solution; orthonasal	sweaty, cheesy	[a] Ferreira et al., 2000; [b] Meilgaard, 1975
Octanoic acid	500	model wine solution; orthonasal	fatty, unpleasant	[a] Ferreira et al., 2000; [b] Escudero et al., 2004
Phenols				
4-ethylguaiacol	33	model wine solution; orthonasal	smoky, gammon-like	[a] Ferreira et al., 2000; [b] Czerny et al., 2008
4-vinylguaiacol	1100	model wine solution; orthonasal	clove-like, smoky	[a] Ferreira et al., 2000; [b] Czerny et al., 2008
Sulphur Compounds				
Dimethyl sulphide	10	hydro-alcoholic solution; retronasal	asparagus-like, putrid	[a] Guth, 1997; [b] Czerny et al., 2008
3-(methylthio)-1-propanol	500	hydro-alcoholic solution; retronasal	cooked potato-like	[a] Guth, 1997; [b] Czerny et al., 2008
Carbonyl Compounds				
Acetaldehyde	10 000	hydro-alcoholic solution; orthonasal	fresh, green	[a] Moreno et al., 2005; [b] Czerny et al., 2008
3-hydroxy-2-butanone	30 000	hydro-alcoholic solution; orthonasal	fruity, moldy, woody	[a] Moreno et al., 2005; [b] Meilgaard, 1975
2,3-butanedione	100	hydro-alcoholic solution; retronasal	buttery	[a] Guth, 1997; [b] Czerny et al., 2008

Table 1. Perception thresholds and odour descriptors of relevant wine volatile compounds

1.2 Varietal compounds

The wine constituents linked to grape variety are the monoterpenols, abundant in Muscat varieties, the methoxypyrazines, which characterize the *Cabernet* family, the C_{13}-norisoprenoids, numerous in *Chardonnay*, volatile thiols in *Sauvignon*, volatile phenols in *Traminer aromatico* and dimethyl sulphide in *Syrah*, but these compounds could also contribute significantly to the aroma of several other varieties (Allen et al., 1991; Sefton et al., 1993; Segurel et al., 2005; Tominaga & Dubourdieu, 2000; Versini, 1985). Except for the methoxypyrazines, these constituents occur in grapes in the form of non-volatile precursors like unsaturated fatty acids, glycosides, carotenoids, cysteine S-conjugates and phenolic acids, which can originate flavour compounds during or after the technological sequence of winemaking (Bayonove et al., 1998). However, monoterpenols are also abundant as free odorants in some grape varieties, like *Muscat* or *Gewürztraminer*.

Monoterpenes are C_{10}-terpenoids which are formed in the plant, by the fusion of two molecules of isopentenylpyrophosphate by the so-called ispoprene-rule, and subsequent enzymatic reaction. Considering flavour properties, monoterpenols are the most interesting terpenoids, namely linalool, HO-trienol, α-terpineol, nerol and geraniol, and two monoterpenic oxides, rose oxide and nerol oxide (Ribéreau-Gayon et al., 2000). These compounds have low perception thresholds, in the range of μg L^{-1}, and may contribute to the floral notes of wines (Table 1). It is well established that the concentration of monoterpenols increase during the maturation period, but the optimum date could be attained before commercial maturation; they are mostly located in grape skin, depending on grape variety however. Consequently, the adopted technology to extract grape juice may have a huge influence in the final wine. Some non-odoriferous monoterpenes may undergo chemical transformations during wine storage and ageing, leading to the appearance of interesting odoriferous monoterpenes. For example, *wine lactone* may be formed from *E*-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid (Bonnländer et al., 1998) and the possible precursors of rose oxide could be 3,7-dimethylocta-5-en-1,7-diol and 3,7-dimethylocta-7-en-1,6-diol (Rapp et al., 1984). On the other hand, monoterpenic polyols (*e.g.* 3,7-dimethylocta-1,5-dien-3,7-diol, 3,7-dimethylocta-1,7-dien-3,6-diol and 3,7-dimethylocta-1-en-3,6,7-triol) may undergo chemical transformations at the acidic conditions present in wines, resulting in low perception threshold monoterpenic compounds (Williams et al., 1980).

Methoxypyrazines are nitrogen heterocyclic compounds originated probably from aminoacids catabolism (Bayonove et al., 1998; Ribéreau-Gayon et al., 2000), although their origin was not completely established. The most referred compounds that influences wine aroma are 3-isobutyl-2-methoxypyrazine, 3-isopropyl-2-methoxypyrazine and 2-methoxy-3-*sec*-butylpyrazine. They contribute to the earthy and vegetable notes – green pepper and asparagus – of wines; the perception thresholds are very low, in the range of some ng L^{-1} (Czerny et al., 2008; Ribéreau-Gayon et al., 2000). Although the methoxypyrazines were detected in *Merlot* and *Semillon* cultivars, they seem to be characteristic of the *Sauvignon* family, namely *Sauvignon blanc* and *Cabernet-Sauvignon* (Allen et al., 1994; Lacey et al., 1991). Methoxypyrazines are located mainly in the skin and their content decrease during grape maturation (Lacey et al., 1991). It must be noted that the "green pepper" character of *Cabernet-Sauvignon* wines could be considered as positive or negative, depending on the concentration of these volatile compounds (Ribéreau-Gayon et al., 2000).

The main volatile thiols identified in wines are 4-mercapto-4-methyl-2-pentanone, 4-mercapto-4-methyl-2-pentanol, 3-mercapto-1-hexenol, 3-mercapto-3-methyl-1-butanol and 3-mercaptohexyl acetate (Tominaga et al., 1996; Tominaga & Dubourdieu, 2000). These compounds are present in grapes in the form of cysteine S-conjugates being enzymatically liberated during winemaking or during tasting of a wine. Vegetal notes –box tree and broom– as well as fruit notes –passion fruit, grapefruit– are associated to these compounds; additionally, perception thresholds are extremely low, of few ng L^{-1} (Table 1). Although these compounds were initially referred as typical of *Sauvignon blanc* wines, the actual knowledge indicates they are widespread among cultivars, *e.g. Gewürztraminer*, *Riesling*, *Pinot blanc*, *Semillon*, *Cabernet-Sauvignon*, *Merlot*, etc. (Tominaga & Dubourdieu, 2000).

C$_{13}$-norisoiprenoids derive from carotenoids, by oxidative degradation (Enzell, 1985), and are usually divided in two groups: megastigmanes and non-megastigmanes. For megastigmanes, ionone series (oxygenation in C$_9$) and damascone series (oxygenation in C$_7$) are reported. These compounds are present in grapes as glycoconjugates, although they could appear episodically in the free form. Nevertheless, they are abundant in wines because glycoconjugates are susceptible of being hydrolysed, enzymatically or in the acidic conditions of wine. The megastigmanes β-ionone and β-damascenone are the most cited C$_{13}$-norisoprenoids, with floral and fruit notes and low perception thresholds (Baumes et al., 1986; Sefton et al., 1993); although β-damascenone is important for the aroma of the majority of white and red wines, β-ionone seems to be only significant on the aroma of some red wines (Etiévant et al., 1983). Two non-megastigmanes are also frequently cited: vitispirane –camphor odour– and mainly 1,1,6-trimethyl-1,2-dihydronaphtalene (TDN), with perception threshold of 20 μg L^{-1}, which contributes to the kerosene and petroleum character of old *Riesling* wines (Simpson, 1978). Some non-megastigmanes derive from megastigmanes by diverse chemical reactions (Sefton et al., 1989). It must be noted, that solar exposition favours the synthesis of carotenoids in grapes before *véraison* and its degradation to C$_{13}$-norisoprenoids after this date (Razungles et al., 1993).

Glycosidic precursors are of greater importance as they can be hydrolysed to a certain extent during winemaking, wine conservation and ageing, chemically or by microorganisms endogenous enzymes, and also by the addition of exogenous enzymes. It makes possible the production of aromatic wines, with varietal characteristics, from non-aromatic varieties (D'Incecco et al., 2004; Günata et al., 1993). Respecting monoterpenic compounds, the glycoconjugated fraction of varietal flavour compounds is usually more abundant than free fraction (Günata et al., 1985; Oliveira et al., 2000). Glycosidic precursors are constituted by a glycone part formed by one or two sugar molecules, linked to a volatile aglycone. The sugars could be β-D-glucopyranoside, 6-0-α-L-rhamnopyranosil-β-D-glucopyranoside (rutinoside), 6-0-α-L-arabinofuranosil-β-D-glucopyranoside, 6-0-β-D-apiofuranosil-β-D-glucopyranoside or, less frequently, α-D-glucopyranoside (Voirin et al., 1990; Watanabe et al., 1997); the volatile aglycones could be monoterpenic alcohols and oxides, C$_{13}$-norisoprenoids, volatile phenols, linear alcohols, etc. (Oliveira et al., 2000). These glycoconjugates could be enzymatically hydrolysed by the action of the enzymes α-rhamnosidade, α-arabinosidase, β-apiosidase and β-glucosidase; in a first step, the first three enzymes hydrolyse disaccharides at the 1,6 linkage releasing rhamnose, arabinose and apiose and then β-glucosidase liberates the volatile aglycon from glucose (Dupin et al., 1992; Günata et al., 1988). Contrarily to varietal volatile compounds in the free form, the

glycoconjugates are more equitably distributed between skin and pulp (Günata et al., 1985; Wilson et al., 1986). Solar exposition favours the synthesis of glycoconjugates which accumulate in grape during the maturation period (Razungles et al., 1998).

Some phenolic acids like caffeic acid, *p*-coumaric acid and ferulic acid can act as precursors of volatile phenols, which could contribute positively to wine aroma, when they are present at low concentrations; associated descriptors are smoky, clove-like and leather (Table 1). Yeasts can conduct the decarboxylation of phenolic acids to volatile phenols, as well as esterase activities present in enzymatic preparations used in winemaking. During wine storage and ageing, volatile phenols may be further transformed.

These varietal compounds were usually used to classify grape varieties in Muscat, aromatic non-muscat and neutral or, at the limit, to discriminate them or even to distinguish *terroirs* (Genisheva & Oliveira, 2009; Marais et al., 1992; Oliveira et al., 2000).

1.3 Pre-fermentative compounds

Pre-fermentative compounds are formed during harvesting, transport, crushing and pressing, as well as during eventual must heating or grape maceration (Cabaroglu et al., 1997; Nicolini et al., 1996). This group comprises C_6-alcohols and C_6-aldehydes derived from grape lipids (linoleic and linolenic acids), in the presence of oxygen, by a sequence of enzymatic reactions (Crouzet et al., 1998). Volatile compounds produced are hexanal, *Z*-3-hexenal, *E*-2-hexenal and the corresponding alcohols (Moio et al., 2004; Ramey et al., 1986). During winemaking, aldehydes are reduced to the respective alcohols by yeasts. Another C_6-compound is usually present in wines, *E*-3-hexenol, but references about its formation mechanism aren´t found. The herbaceous flavour characterise this group of compounds.

1.4 Fermentative compounds

Fermentative compounds are alcohols, fatty acids, esters, carbonyl compounds, sulphur compounds and some volatile phenols (Bayonove et al., 1998); they contribute to the vinous character of wine and are, quantitatively, the majority of volatile compounds. These compounds are produced mainly during alcoholic fermentation and the minor part, but not less important, if it occurs, during malolactic fermentation. Therefore, all wines present a similar pattern of fermentative compounds.

Alcohols having more than two carbons and only one alcohol function are usually named higher alcohols. They are practically absent in grapes and musts but they are found in wines at relatively higher concentrations, reaching together values greater than 100 mg L^{-1}. The main alcohols are 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol (isoamyl alcohol) and 2-phenylethanol, frequently above 50 mg L^{-1}, and 1-propanol, 1-hexanol, between 1 mg L^{-1} and 50 mg L^{-1}; another frequently cited alcohol, cooked potato-like odour, is 3-(methylthio)-1-propanol also known as methionol (Czerny et al., 2008; Ferreira et al., 2000). However, only 2-methyl-1-butanol, 3-methyl-1-butanol, 2-phenylethanol and methionol seem to contribute to wine aroma. Nevertheless, some authors report about 300 mg L^{-1} as the upper limit at which the overall concentration contribute positively to wine aroma (Rapp & Mandery, 1986; Rapp & Versini, 1995); this value depends on the rest of composition, however. The formation of higher alcohols is linked to the amino acids

metabolism by yeasts, through the Erhlich mechanism (catabolic pathway) where amino acids undergo successively a deamination, a decarboxylation and a reduction; they could also be synthesised through the metabolism of the sugars (anabolic pathway), via pyruvate, having the keto acids as intermediates. The contribution of each of these pathways depends on the higher alcohol and on the yeast assimilable nitrogen present in the medium (Henschke & Jinarek, 1993).

Volatile fatty acids present in wine may derive from the anabolism of lipids, resulting in compounds with even number of carbon atoms, by oxidative decarboxylation of α-keto acids or by the oxidation of aldehydes. Volatile fatty acids synthesised from α-keto acids are mainly propanoic acid, 2-methyl-1-propanoic acid (isobutyric acid), 2-methyl-1-butanoic acid, 3-methyl-1-butanoic acid (isovaleric acid; 3-methylbutyric acid) and phenylacetic acid. From lipid metabolism, the following fatty acids are reported: butanoic acid (butyric), hexanoic acid (caproic), octanoic acid (caprylic) and decanoic acid (capric) (Dubois, 1994). Although fatty acids are characterized by unpleasant notes (Table 1), only few compounds of this family attain its perception threshold. However, their flavour is essential to the aromatic equilibrium of wines (Etiévant, 1991).

Ethyl esters are formed from the reaction between ethanol and fatty acids, while acetates results from the esterification of a higher alcohol with acetic acid. Esters present, generally, fruity pleasant flavours, except ethyl acetate which is not well accepted at concentrations above 100 mg L^{-1}. Although, as longer is the chain, lesser pleasant is the volatile ester (Table 1). As olfactory notes are similar, a synergic effect is reported (Dubois, 1994). The synthesis of esters is dependent on the need of yeasts to form fatty acids, which is also correlated with the amount of assimilable nitrogen in must (Bell et al., 1979; Nykänen, 1986). There are a huge number of esters found in wines since, in theory, each fatty acid may react with each alcohol to form an ester. Among them, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, 3-methylbutyl acetate (isoamyl acetate) and 2-phenylethyl acetate are the main contributors to the aroma of young wines (Dubois, 1994; Oliveira et al., 2008a). For this group of compounds, the reported synergic effect may reduce individual perception threshold (Dubois, 1994); for this reason, other esters such as hexyl actetate, 3-methylpropyl acetate (isobutyl acetate) may also contribute to the fruity character of wines. Esters of fixed acids like diethyl succinate and ethyl lactate are present at higher levels in wines but, since their perception thresholds are also very high, only ethyl lactate may contribute, occasionally, to wine aroma (Dubois, 1994).

Sulphur compounds produced by yeasts are mainly thiols, mono and polysulphides, and thioesters. They are regularly associated with powerful and undesirable odours; nevertheless, as molecular weight increases, the negative perception is reduced. Accordingly, two distinct groups are usually reported: low molecular weight sulphur compounds (boiling point < 90 ^{o}C) and high molecular weight sulphur compounds (boiling point > 90 ^{o}C). The first group is usually related to organoleptic defects while the second one participates on wine aroma in a very complex way (Darriet et al., 1999; Dubois, 1994; Etiévant, 1991). Light sulphur compounds present at the end of alcoholic fermentation apart from sulphur dioxide are methyl mercaptans (*e.g.* methanethiol), ethyl mercaptans (*e.g.* ethanethiol) and respective thioacetates, sulphides (*e.g.* hydrogen sulphide, carbonyl sulphide) and disulphides (*e.g.* carbon disulphide). Although these compounds are associated to bad descriptors like rotten eggs, onion and rubber, they could participate

positively to wine aroma if the concentration is near de perception threshold (Bayonove et al., 1998; Darriet et al., 1999). Main heavy sulphur compounds are secondary products of amino acids metabolism (cysteine, methionine and homomethionine). The most common cited are 2-mercaptoethanol, 2-methylthioethanol, 3-(methylthio)-1-propanol (methionol), methionyl acetate, methional and 3-methylthiopropanoic acid.

A vast number of carbonyl compounds could be formed by α-keto acids decarboxylation. Nevertheless, as they are reduced by yeasts and/or by the presence of SO_2, they exist in wines at levels which are not easily detectable. The compounds susceptible of influencing wine aroma are basically acetaldehyde, 3-hydroxy-2-butanone (acetoin) and 2,3-butanedione (diacetyl) (Bayonove et al., 1998).

1.5 Post-fermentative compounds

It is well known that during wine storage and ageing there are many chemical changes in the volatile composition. These reactions depend on wine composition, pH, storage time and temperature (Marais & Pool, 1980; Usseglio-Tomasset, 1983). The majority of fatty acid ethyl esters is hydrolysed during conservation and, ethyl esters of fatty acids related to yeast nitrogen metabolism (*e.g.* ethyl 2-methylbutyrate and ethyl 3-methylbutyrate) and esters of organic acids (*e.g.* diethyl succinate) increase during this period (Díaz-Maroto et al., 2005; Dubois, 1994; Oliveira et al., 2008a); however, diethyl succinate do not influence wine aroma. Also, the terpenic profile may change, with the disappearance or strong decline of the compounds initially present, with the simultaneous formation of other terpenic compounds with higher oxidation state; temperature and pH have a decisive influence (Di Stefano & Castino, 1983; Marais et al., 1992). Dimethyl disulphide increases during wine storage (Marais & Pool, 1980). Some norisoprenoids may appear or increase their concentration during the ageing period, *e.g.* β-damascenone, TDN and vitispirane (Marais et al., 1992). The acidic medium also favours the hydrolysis of glycosidic precursors and the transformation of aglycon moieties (Dugelay, 1993; Sefton et al., 1993).

2. Extraction of grape and wine volatiles

When dealing with grapes, a blender is usually used to liquefy the matrix, being the analytes dissolved from pulp and skin into the must. Particle size of the fragmented skin can be an important parameter to obtain reproducible results as the extent to which the grapes are broken up can influence the extraction rates. Furthermore, prior to extraction, the must should be clarified by centrifugation and filtration to assure a clean final solution (Oliveira el al., 2008a). Respecting wines, only the clarification step should be implemented if necessary. After this initial step, samples (grape juices, musts and wines) could be treated as liquid samples.

The wine volatile fraction is extremely complex, mainly because of the great number of compounds, which are from different chemical classes, covering a wide range of polarities, solubility and volatilities. Moreover, the concentration range of these compounds is from a few ng L^{-1} to hundreds of mg L^{-1}. Furthermore, volatile compounds are contained in complex and compositionally very variable matrices where they can be associated and therefore their volatility modulated by other wine macro-components (polyphenols, ethanol, polysaccharides) (Andujar-Ortiz et al., 2009; Pozo-Bayón & Reineccius, 2009). Finally, but

also of importance, is the fact that many aroma compounds are chemically very unstable and can be easily oxidized or thermo degraded (Castro et al., 2008). Although grape volatile composition is simpler than that of wines, analyses may deal with small concentrations, equal or below the µg L⁻¹ level.

Accordingly, the determination of volatile compounds in grapes and wines often requires extensive sample extraction and preparation regimes prior to instrumental analysis. The amount of sample preparation needed depends on the sample matrix and the properties and level of analyte to be determined (Ridgway et al., 2007). The typical steps within sample preparation include sampling/homogenisation, extraction, clean-up and concentration followed by the final analysis. Another step that can be included at several points is derivatisation. For the determination of volatile compounds, the final analysis is invariably achieved using a powerful separation technique, gas chromatographic, combined with an appropriate detector (Ridgway et al., 2007).

There are available a wide range of analytical tools for the extraction of volatile compounds. These methodologies are essentially based on the solubility of the analytes in organic solvents, based on the adsorptive capacity of polymeric phases and based on their sorptive capacity on polymeric phases or solvents. Moreover, since the techniques deal with volatile compounds, the headspace approach is typically associated whenever appropriate (Andujar-Ortiz et al., 2009; Ridgway et al., 2007).

Currently, the most commonly used methods are *liquid-liquid extraction* (LLE), *solid phase extraction* (SPE) and *solid phase microextraction* (SPME). LLE is a versatile technology which may possibility simultaneous extractions using solvents with distinct polarities; since earlier works used large solvent volumes, the tendency to reduce costs and environmental impacts conducted to *liquid-liquid micro extraction* (LLME) and *dispersive liquid-liquid micro extraction* (DLLME). SPE also uses diminutive volumes of solvent and as the advantage of being a selective technique by using appropriate adsorbent phases. The SPME technique, as well as *stir bar sorptive extraction* (SBSE), is a solvent-free approach with very low limits of detection; SPME, mainly, starts to dominate the field by replacing the ancient methods. Applications of the most common methodologies used for the extraction of grape and wine volatiles are illustrated in Table 2.

Other two methodologies are described in literature: *supercritical fluid extraction* (SFE) and *liquid-phase microextraction* (LPME). SFE was successfully applied, using CO_2 or methanol-modified CO_2, respectively to analyse wine volatile compounds (Blanch et al., 1995; Karásek et al., 2003) and to extract grape glycosides (Palma et al., 2000). LPME is an alternative miniaturized sample preparation approach which makes use of only a very small amount of solvent for concentrating analytes (Liu & Dasgupta, 1996; Jeannot & Cantwell, 1997). It overcomes many of the disadvantages of LLE as well as some of those of SPE (*e.g.* independence of a commercial source and sample carryover). Extraction normally takes place between a small amount of water-immiscible solvent (*e.g. n*-octanol, ethylene glycol) and the bulk aqueous phase containing the analytes of interest. The volume of the receiving phase is in the microliter or submicroliter range conducting to high enrichment factors. Since the extraction medium is in the form of a single drop at the tip of a micro-syringe needle, this type of LPME has been termed *single-drop microextraction* (SDME). In the case of

Application of Gas Chromatography on the Evaluation of
Grape and Wine Aroma in Atlantic Viticulture (NW Iberian Peninsula)

119

Extraction technique	Matrix/Analytes	Method	*LOD/* ($\mu g\ L^{-1}$)	Reference
Solvent-based				
LLE	Wine, 52 volatile compounds	50 mL wine, 2×5 mL CH_2Cl_2; extract final volume = 500 µL; GC-MS	2 to 534	Perestrelo et al., 2006
	Synthetic wine, 30 volatile compounds	50 mL wine, 10 mL CH_2Cl_2; extract final volume = 300 µL; GC-MS	1.0 to 34.1	Andujar-Ortiz et al., 2009
	Wine and grapes, β-ionone	100 mL wine (250 mL grape juice), 10 mL CH_2Cl_2; extract final volume = 300 µL; GC-MS		Kotseridis et al., 1999
	Wine, 44 volatile compounds	100 mL wine, 50 mL diethyl ether-*n*-pentane; 30 min sonication, 25 ºC (under N_2); GC-MS		Hernanz et al., 2008
LLME	Wine, 3 C_6-compounds	8 mL wine, 400 µL CH_2Cl_2; 15 min; GC-FID and GC-MS	3.3	Oliveira et al., 2006
	Wine, 35 volatile compounds	8 mL wine, 400 µL CH_2Cl_2; 15 min; GC-FID and GC-MS		Vilanova et al., 2009
	Wine, 40 volatile compounds	3 mL wine + 7 mL H_2O, 200 µL CH_2Cl_2; 2500 min^{-1}, 10 min; GC-FID		Ortega et al., 2001
DLLME	Wine, 5 chlorophenols and 7 haloanisoles	5 mL wine, 1 mL acetone (disperser) + 30 µL CCl_4 (extraction solvent); GC-MS	0.004 to 0.108	Campillo et al., 2010
	Wine, 8 volatile compounds (cork and Brett taints)	5 mL wine, 1.43 mL acetone (disperser) + 173 µL $CHCl_3$ (extraction solvent); GC-MS/MS	0.05 to 0.75	Pizarro et al., 2011
	Wine, geosmin and 2-methylisoborneol	12 mL wine, 8 µL C_2Cl_4; ultrasound 3 min, 20 ºC; GC-MS	0.002 to 0.009	Cortada et al., 2011
Adsorptive-based				
SPE	Synthetic wine; 30 volatile compounds	50 mL wine, LiChrolut-EN cartridge (200 mg), 1.3 mL CH_2Cl_2; GC-MS	0.1 to 46.5	Andujar-Ortiz et al., 2009
	Wine; glycosylated volatile compounds	15 mL wine; LiChrolut-EN (200 mg), Amberlite® XAD-2 (280 mg) and Lichrolut RP-18 (200 mg) cartridges; 4 mL pentane-dichloromethane (2 :1); 7 mL ethyl acetate; final volumes = 200 µL; GC-MS		Ibarz et al., 2006
	Wine; free and glycosylated terpenes	25 mL wine; C-18 column; 35 mL dichloromethane; 30 mL methanol; final volumes = 1 mL		Karagiannis et al., 2000

Extraction technique	Matrix/Analytes	Method	$LOD/$ $(\mu g\ L^{-1})$	Reference
	Wine; volatile compounds	100 mL wine; 10 mL Amberlite® XAD-2 bed; 50 mL pentane-dichloromethane (2:1), final volume = 2 mL; 50 mL ethyl acetate, final volume = 200 µL; GC-MS	0.3	Oliveira et al., 2008b
	Grapes; free and glycosylated volatile compounds	150 mL grape juice; 10 mL Amberlite® XAD-2 bed; 50 mL pentane-dichloromethane (2:1), final volume = 200 µL; 50 mL ethyl acetate, final volume = 200 µL; GC-MS	0.1	Genisheva & Oliveira, 2009
Sorptive-based				
SPME	Synthetic wine; 30 volatile compounds	8 mL wine, StableFlex 85 µm CAR-PDMS fibre, 5000 min⁻¹; GC-MS	1.1 to 270.4	Andujar-Ortiz et al., 2009
	Grape juice medium; 9 off-flavours	10 mL grape juice medium, pH = 3.4, NaCl = saturation; DVB/CAR/PDMS fibre, HS-SPME, 30 min, 50 °C; GC-MS	0.0047 to 3	Morales-Valle et al., 2010
	Wine; 34 volatile compounds	40 mL wine, 50 mL vial; DVB/CAR/PDMS fibre, HS-SPME, 15 min, 37 °C; GC-FID and GC-MS		Tat et al., 2005
	Wine, 8 halophenols and haloanisoles	10 mL wine, 20 mL vial; PA fibre, HS-SPME, 60 min, 70 °C; derivatization with MSTFA 25 °C, 25 min; GC-ECD	0.0004 to 0.0038	Pizarro et al., 2007
SBSE	Grape juice, 34 volatile compounds	100 mL prepared juice; PDMS bar (10 mm × 0.5 mm), SBSE; 6 h, room temperature; TD 290 °C, 4 min, trap = - 30 °C; GC-MS		Salinas et al., 2004
	Wine; 13 oak related volatiles	25 mL wine; PDMS bar (10 mm × 0.5 mm), SBSE; 90 min, room temperature, 700 min⁻¹; TD 290 °C, 4 min, trap = - 30 °C; GC-MS	0.0001 to 38.93	Marín et al., 2005
	Wine; 39 volatile compounds	0.5 mL wine, 1.5 g NaCl, 20 mL vial; PDMS bar (10 mm × 0.5 mm), HSSE; 1 h, 23 °C, 1200 min⁻¹; TD; GC-MS		Weldegergis & Crouch, 2008

Table 2. Most common methodologies used for the extraction of grape and wine volatiles (*LOD* – limit of detection)

volatile compounds, the acceptor phase is usually suspended above the sample (headspace extraction; HS-SDME), but direct immersion (DI-SDME) either in static or in dynamic mode is also applicable. The operation of this technique is somewhat similar of SPME, being the syringe, after the extraction procedure, transferred to the injection port of a gas chromatograph (Xu et al., 2007). An alternative concept of LPME is based on the use of a single, low-cost, disposable, porous, hollow-fibre made of polypropylene – HF-LPME (Rasmussen & Pedersen-Bjergaard, 2004). In this hollow fibre-based LPME device, the micro-extract is contained within the lumen of a porous hollow fibre, so the micro-extract is not in direct contact with the sample solution. As a result, samples may be stirred or vibrated vigorously without any loss of the micro-extract. Several applications of LPME were published concerning food analysis (Asensio-Ramos et al., 2011), but the majority refers to the extraction of non-volatile analytes (*e.g.* fungicides and pesticides). In spite of the possibilities of this technique, there are reported only few papers respecting volatile compounds. However, the extraction of the taints 2,4,6-trichloroanisole and 2,4,6-tribromoanisole in wines (Márquez-Sillero et al., 2011; Martendal et al., 2007), and alcohols and volatile sulphur compounds in beer (Tankeviciute et al., 2001; Xiao et al., 2006) should be referred.

2.1 Solvent-based techniques

In *liquid-liquid extraction* (LLE), analytes are extracted by direct partitioning with an immiscible solvent. This technique is based on the relative solubility of an analyte in two immiscible phases and is governed by the equilibrium distribution/partition coefficient. Extraction of an analyte is achieved by the differences in solubilizing power (polarity) of the two immiscible liquid phases. Typically a separating funnel is used and the two immiscible phases are mixed by shaking and then allowed to separate. To avoid emulsions, in some cases, salt may be added and centrifugation can be used if necessary. Alternatively a *matrix solid phase dispersion* (MSPD) approach can be used to avoid emulsions. Either layer can be collected for further analysis. To ensure the complete extraction of an analyte into the required phase, repeated extractions may be necessary. The major disadvantage of bulk liquid–liquid extraction is the need for large volumes of organic solvents. Also, due to the limited selectivity, particularly for trace level analysis, there is a need for clean-up or analyte enrichment/concentration steps prior to instrumental analysis (Ridgway et al., 2007). Although LLE is being replaced by more manageable and solvent-free techniques, this type of extraction is still a reference for the analysis of wine aroma compounds (Andujar-Ortiz et al., 2009; Kotseridis et al., 1999; Perestrelo et al., 2006; Cabredo-Pinillos et al., 2006; Hernanz et al., 2008; Jofré et al., 2010). Organic phases commonly used are dichloromethane, freon-11, *n*-pentane, diethyl ether, hexane–diethyl ether and diethyl ether–*n*-pentane, among others. The main advantages of this technique are its capacity to extract a wide range of compounds of different volatilities (as long as they have an affinity to the solvent), the high repeatability and the possibility of carrying out simultaneous extractions (Andujar-Ortiz et al., 2009). Table 2 show some examples of application of LLE technique.

Some variants of LLE technique were also referred to extract grape and wine volatile compounds. *Simultaneous distillation-extraction* (SDE) was applied to extract volatile compounds of grape juice (Caven-Quantrill & Buglass, 2006) and wine (Blanch et al., 1996). Carro et al. (1997) used *microwave-assisted extraction* (MAE) to analyse monoterpenes in must

samples. Also, *ultrasound-assisted extraction* (UAE) was successfully applied to must and wine samples (Cabredo-Pinillos et al., 2006; Peña et al., 2005; Hernanz et al., 2008; Cocito et al., 1995).

The necessity to reduce solvent volumes and therefore the costs, as well as the consumable time, conducted to the miniaturization of LLE techniques. In this way, the *liquid-liquid microextraction* – LLME – technique (Ortega et al., 2001; Oliveira et al., 2006) corresponds to a direct miniaturization of LLE, employing only a few hundreds of μL of solvent and a few mL of wine (table 1); the extraction is obtained by stirring the mixture in a sealed glass tube during about 10 min to 15 min; then the organic phase is detached by centrifugation before being collected by a syringe or a Pasteur pipette. This approach permits multiple parallel extractions, in short time period, using ordinary labware, and restricting the use of toxic solvents.

Recently, a novel and powerful microextraction technique, named *dispersive liquid–liquid microextraction* (DLLME) was suggested (Rezaee et al., 2006). In this method, the appropriate mixture of extraction solvent and disperser solvent, the extractant, is injected into the aqueous sample by a syringe, rapidly. This turbulent regimen gives rise to the formation of small droplets, which are dispersed throughout the aqueous sample, with very large interfacial area. Thereby, a cloudy solution is formed and the equilibrium state is achieved quickly and, therefore, the extraction time is very short (Rezaee et al., 2010). In fact, this is the principal advantage of DLLME. After centrifugation of the cloudy solution, a sedimented phase is settled in the bottom of a conical tube and used with the most appropriate analytical technique. Other advantages of DLLME include simplicity of operation, rapidity, low cost, high recovery and high enrichment factor. Campillo et al. (2010) and Pizarro et al., (2011) applied the technique to analyse some compounds responsible for the cork taint and the *Brett* character of wines, namely chlorophenols, chloroanisoles and volatile phenols. The use of ultrasound energy to disrupt the extractant phase may reduce even more the consumption of organic solvent because the disperser solvent is not needed. It is the achievement of the new *ultrasound-assisted dispersive liquid–liquid microextraction* (Cortada et al., 2011).

2.2 Adsorptive-based techniques

Solid phase extraction (SPE) involves a liquid–solid partition, where the extracting phase is a solid sorbent and it has been used extensively to remove and concentrate trace organic materials from liquid samples or solutions. The possibility of using different sorbent phases and eluents makes SPE a very selective technique, using different mechanisms for extraction/retention of analytes. A change in pH can be used to enable extraction. A wide range of sorbents have been used including C_8 and C_{18} bonded phases on silica, polymeric resins (polystyrene/divinylbenzene copolymer), Florisil (activated magnesium silicate), polar sorbents such as alumina, charcoal, silica and cyano and amino-bonded. Ionic functional groups, such as carboxylic acid or amino groups can also be bonded to silica or polymeric sorbent to create ion-exchange sorbents. Mixed-mode sorbents are also available using both the primary and secondary mechanisms for selective retention of analytes; also, specific selective sorbents have been designed. These different phases enable interactions based on adsorption, H-bonding, polar and non-polar interactions, cation, anion exchange or size exclusion (Castro et al., 2008; Ridgway et al., 2007).

SPE has been extensively used to analyse grape and wine volatiles, either in the form of resin cartridges or even in hand-prepared bed columns. The most used sorbents are C_{18} bonded phases, polystyrene/divinylbenzene copolymers and hydrophobic cross-linked polystyrene copolymer resins under the commercial names Bond Elut C18, Discovery DSC-18, Strata® C18, Lichrolut® RP-18, Lichrolut® EN, Chromabond® easy, Strata® SDB-L and Amberlite® XAD-2, among others.

This technique has been used to extract volatile compounds of wines either indistinctly or selectively, *e.g.* polyfunctional mercaptans, methoxypyrazines and terpenoids (Culleré et al., 2003; López et al., 2002, 2011; Mateo-Vivaracho et al., 2009; Weldegergis et al., 2011). However, its major advantage is the application to determine the nature and concentration of free and glycosidically bound volatile compounds in grape juices and wines. In summary, the methodology initially proposed by Günata et al. (1985), involves a previous activation of the resin (*e.g.* successively few mL of methanol and hydroalcoholic solution), followed by the percolation of about 25 mL to 150 mL of the liquid sample previously spiked with a known amount of an internal standard; then, the resin is washed with water and the free volatile compounds are extracted with few mL of an appropriate solvent (*e.g.* dichloromethane or azeotropic mixture pentane-dichloromethane 2:1, v/v); after that, the glycosidically bound volatile compounds are eluted with few mL of another solvent, *e.g.* ethyl acetate, methanol or ethyl acetate-methanol mixture 9:1 (Canosa et al. 2011; Genisheva & Oliveira, 2009; Ibarz et al., 2006; Oliveira et al., 2008b; Schneider et al., 2004). The extract containing glycosylate volatile compounds should be further treated. The solvent is removed and the glycosides ressuspended in an appropriate buffer before enzymatic or acidic hydrolysis; the liberated volatile compounds are then extracted with the same solvent used for free volatile fraction. Finally, the extracts are concentrated to about 100 µl to 200 µL in the case of grape juices and musts; when analysing wines, depending on the compounds of interest, the "free" extract may be concentrated to 1 mL, only.

Recently, a novel methodology has been developed which corresponds to a miniaturization of the conventional SPE (Abdel-Rehim, 2010; Altun et al., 2004). It was named *microextraction in packed syringe* (MEPS) and the major achievement is the considerably reduction of sample, as well as the extraction and washing solvent volumes. MEPS consist of two parts: the syringe and the barrel insert and needle assembly containing the SPE phase. The extraction phase of MEPS is based on a double pass system where the sample solvent both enters and exits from the bottom of the same bed volume. After extraction the bed volume can be washed with solvent before elution of the target compounds. However, until now, the application of this methodology to wine volatile compounds is limited to the analysis of the taints 2,4,6-tricholoanisole and 2,4,6-tribromoanisole (Jönsson et al., 2008).

2.3 Sorptive-based techniques

Sorptive extraction techniques are based on the distribution equilibria between the sample matrix and a non-miscible liquid phase. Matrices are mostly aqueous and the non-miscible phase is often coated onto a solid support. Analytes are extracted from the matrix into the non-miscible 'extracting' phase. Unlike adsorption techniques, where the analytes are bound to active sites on the surface, the total volume of extraction phase is important. Extraction of analytes depends on the partitioning coefficient of solutes between the phases (Ridgway et al., 2007). Two extraction techniques are commonly employed: *solid phase microextraction* (SPME) and *stir-bar sorptive extraction* (SBSE).

SPME is a solvent free sample preparation technique, originally developed by Pawliszyn and co-worker (Arthur & Pawliszyn, 1990). The initially developed fibre-SPME device continues to be the most widely used format. It consists of a fibre holder and a fibre assembly, the later containing a 1 cm to 2 cm long retractable fibre. The SPME fibre itself consists of a thin fused silica fibre coated with thin polymeric coating. The process involves the performance of two basic steps: (i) partitioning of analytes between the extraction phase and the sample matrix and then (ii) desorption of the concentrated extracts from the fibre into the analytical instrument, *e.g.* a gas chromatograph (Risticevic et al., 2009). The utilization of this technique offers the following benefits: short sample preparation times; small sample volumes; analyte concentration from liquid, gaseous and solid samples; solvent-free technique and; easily automated to allow the high-throughput analysis. The complete automation, one of the initially objectives of the invention, could achieve by the use of a commercial autosamplers (*e.g.* CombiPAL™, TriPlus™).

Generally SPME extraction of the analyte from the matrix is not an exhaustive extraction technique but is an equilibrium technique. The maximum sensitivity is obtained at the equilibrium point; however, it is not necessary to reach this point and the extractions can instead be performed for a defined period of time (Kataoka et al., 2000; Risticevic et al., 2009). When developing a SPME method, the following parameters should be optimized: type of coating; sampling mode (direct immersion or headspace); agitation conditions (speed, time and temperature); ionic strength; pH; sample volume; vial shape and headspace volume and; desorption conditions (Pawliszyn, 2009). The presence of high concentrations of matrix components or other compounds can result in competitive binding and displacement and potentially large errors can occur. Therefore, matrix effects can be an issue and quantitation should consider the use of the method of standard additions or the use of an isotopically labelled internal standard.

Currently, various types of stationary phases are commercially available, with different thickness and polarities, showing different affinities for different analytes. The commercially available single coatings and blending materials, sorting for increasing order of polarity, are: PDMS, CAR/PDMS (CAR = carboxen), DVB/CAR/PDMS (DVB = divinylbenzene), PA, PDMS/DVB, CW/DVB (CW = carbowax = polyethylene glycol) and CW/TPR (TPR = templated resin). The apolar phase PDMS presents affinity for apolar compounds (*e.g.* esters) and the polar phase PA is appropriate for polar compounds (*e.g.* alcohols). Furthermore, coatings utilizing the sol-gel technology have been developed (Kataoka et al., 2000; Liu et al., 2005). Recently, Ho et al. (2011) proposed the application of ionic liquids (ILs) and polymeric ionic liquids (PILs) as sorbent materials for SPME. Because of their unique physico-chemical properties, these compounds can be structurally-designed to selectively extract target analytes based on unique molecular interactions.

The need to use high throughput applications requires robust and reliable SPME assemblies. However, some disadvantages of SPME include batch to batch variation and robustness of fibre coatings which could only be used for 50 to 100 cycles (Risticevic et al., 2009). To overcome this problem, a special type of insert, flexible nickel-titanium alloy was used to construct the assembly needle, fibre core and plunger components of the metal SPME assembly. These materials permit more than 600 extraction/desorption cycles (Giraudel et al., 2007; Setkova et al., 2007a, 2007b).

More than one hundred papers reporting diverse applications to analyse wines were published until know. The majority of the referred methodologies use the *headspace* mode (HS-SPME) instead of the *direct immersion* mode (DI-SPME). In terms of performance, SPME showed comparable results to LLE or SPE. However, SPME is simpler and solvent-free, and uses smaller volumes of sample; nevertheless, on the other hand, LLE had the possibility of carrying out simultaneously the extraction of several samples (Bohlscheid et al., 2006; Castro et al., 2008). When the interest is to obtain the maximum information about the volatile fraction of a wine, the coating DVB/CAR/PDMS seem to be the most suitable (Tat et al., 2005). On the other hand, for specific applications, the choice of a suitable solid-phase, depends on the class of compounds be analyzed, *e.g.* CAR/PDMS for volatile sulphides and disulphides (Mestres et al., 1999), on-fibre derivatization (PA) for the determination of haloanisoles and halophenols (Pizarro et al., 2007).

Respecting grape juices and musts, the number of published works is much lower. For example, Sánchez-Palomo et al. (2005) studied 16 varietal compounds of grapes by HS-SPME and Morales-Valle et al. (2010) have determined the concentration of geosmine and fungal "off" volatiles metabolites in musts inoculated with *Botrytis cinerea* and *Penicillium expansum*.

Stir bar sorptive extraction (SBSE) was introduced in 1999 (Baltussen et al., 1999) as a solventless sample preparation method for the extraction and enrichment of organic compounds from aqueous matrices. In this sorptive-based method, the solutes are extracted into a polymer coating (polydimethylsiloxane – PDMS) on a magnetic stirring rod. Stir bar sorptive extraction of a liquid sample is performed by placing a suitable amount of sample in a vial. The stir bar is added and the sample is stirred, typically for 30 min to 240 min. The extraction time is controlled kinetically, determined by sample volume, stirring speed, and stir bar dimensions and must be optimized for a given application (David & Sandra, 2007). Stir bars of 1 cm to 2 cm long coated with 500 μm to 1 mm PDMS have been commercially available. Sampling could be also carried out in the headspace mode (*headspace sorptive extraction* – HSSE), placing the stir bar above the liquid sample using a special devices (David & Sandra, 2007).

After extraction, the stir bar is removed, dipped on a clean paper tissue to remove water droplets, and introduced in a thermal desorption unit (TD). In some cases, it is recommended to rinse the stir bar slightly with distilled water to remove adsorbed sugars, proteins, or other sample components. Alternatively, liquid desorption (LD) can be used, by placing the stir bar, typically, in a small vial (2 mL, or vial with insert) being desorption performed with apolar solvents (*e.g.* hexane). The thermal desorption system is used for optimum desorption and re-concentration before GC analysis. Thermal desorption temperatures between 150 ºC and 300 ºC are typically used. But, because more sorptive extraction phase is used, the desorption process is slower than for a SPME fibre. Longer desorption times (10 min) in combination with desorption flows between 10 mL/min and 100 mL/min are typically used. A programmed-temperature vaporizing (PTV) injector is operated as a cryotrap for cryogenic refocusing of the thermally desorbed analytes. Temperatures as low as −150 ºC are used along with liquid nitrogen cooling (David & Sandra, 2007).

SBSE have large surface area of stationary phase than SPME, leading to a higher phase ratio and hence a better recovery and higher sample capacity. Typically, the coated volume layer is 50 to 250 times larger. Consequently, the extraction efficiency for solutes that are partially water soluble, *i.e.* polar compounds, is much better (David & Sandra, 2007). Typically, the life-time of a single stir bar is 20 to more than 50 extractions, depending on the matrix.

SBSE presents a series of advantages over the rest of extraction techniques: is solvent-free (environmental friendly); could be completely automated; don't requires pre-treatment of samples (reduces analytical errors) and; presents greater sensitivity than SPME, reaching lower detection and quantitation limits. However, it presents two clear disadvantages compared with the other extraction techniques: PDMS is the only phase commercially available to date, limiting the extraction of polar substances and; a specific thermal desorption unit is required for optimize the process (Castro et al., 2008). The increase of the extraction yields for the recovery of polar compounds could be carry out by in-site derivatization. Recently, other phases under development were referred, namely those based on the sol-gel technology, restricted access materials and molecular imprinted polymers (Prieto et al., 2010).

The number of applications to enological products is lower than SPME. However, due to the lower limits of detection achieved with SBSE, the number of published works has largely increased recently. Works refer to analysis of volatile compounds, indistinctly, in wines (Coelho et al., 2009; Fang & Qian, 2006; Tredoux et al., 2008) or grapes (Caven-Quantrill & Buglass, 2006; Salinas et al., 2004). Applications to particular classes of wine volatile compounds include volatile phenols (Díez et al., 2004), terpenes, C_{13}-norsprenoids and C_6-compounds (Zalacain et al., 2007), haloanisoles and halophenols (Zalacain et al., 2004) and related-oak volatiles (Marín et al., 2005). Luan et al. (2005 and 2006) used SBSE to study the metabolism of geraniol in grape berry and to carry out an enantioselective analysis of monoterpenes in different grape varieties during berry ripening. Weldegergis & Crouch (2008) refer the use of HSSE to analyse volatile compounds.

3. Analyses of volatile extracts

Once the volatile compounds were extracted, they should be analyzed by gas-chromatography (GC). Depending on the characteristics of the extracts (solvent, analytes of interest), the GC conditions must be select and optimized. The chromatographic system is composed by an injector, a column inside an oven and a detector. A carrier gas is needed to transport the volatile compounds through the column; depending on the detector, other gases may be required. Finally, an acquisition system collects the information that arrives to the detector.

Some wine volatile compounds could be analysed, however, without previous extraction. Indeed, acetaldehyde, ethyl acetate, methanol and higher alcohols (1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol) could be directly analysed by injecting a filtered wine spiked with a suitable internal standard (*e.g.* 4-nonanol), in the split mode; in that case, the column stationary phase should tolerate the reception of vaporized aqueous samples, *e.g.* CP-Wax 57 CB (100 % chemically-bonded polyethylene glycol).

The final goal of an analytical method is to determine quantitatively each compound of interest. Furthermore, the method must be robust, accurate and reproducible. To achieve these goals, the analytes should ideally appear at the end of the column (detector) as individual peaks to be easily identified and quantified.

3.1 Isolation of volatile compounds by gas chromatography

Sample introduction is made by injecting 1 μL to 5 μL, commonly, by a micro-syringe into the injection port of the injector. Two types of injectors are commonly used: split/splitless injector and programmed-temperature vaporizing injector (PTV). In the split mode, only a small fraction of the sample enters the chromatographic column by splitting the gas flow (*e.g.* 1 % to 6 %); the rest is vented through the split outlet. This mode is used for highly concentrated samples in order to avoid system overloading and when sensitivity is not an issue. In the splitless mode on the other hand, in order to increase the sensitivity, the split valve is closed for a short period of time (*e.g.* 30 s to 2 min) after injection, ensuring that the entire sample is transferred for analysis (Grob & Barry, 2004). In a PTV inlet analytes are trapped at reduced temperature which commonly ranges between -150 ºC and -50 ºC; therefore, it could reduce analyte discrimination during the injection step and promote better recovery of thermo-labile compounds. The PTV inlet could also operate both in split and splitless modes.

The capillary column is the core of a chromatographic separation. Separation occurs based on the physical and chemical properties of each analyte in the sample in relation to the stationary phase of the column. There are a wide range of capillary columns available nowadays, mainly differing in the type of their stationary phases and dimensions. The choice depends mainly on the type and number of analytes to be separated and the complexity of the sample. In a non-polar stationary phase, the separation is made according to boiling point. On the contrary, the separation of polar compounds should be achieved by a polar phase mainly due to selective partitioning. The most extensively used stationary phases are polyethylene glycol –PEG, also known as Wax– and polydimethylsiloxane– PDMS– (Ferreira & Cacho, 2009; Grob & Barry, 2004). Other polyethylene glycol based phases, differing slightly in polarity, are also adopted; for example, FFAP phases are mostly used to analyse volatile fatty acids and phenols. The length, internal diameter and film thickness (d_f) of the column are other three parameters to be chosen. A longer column will give better separation but leads to a longer analysis time. Narrow bore columns will improve separation efficiency and thicker films will provide better sample loading capacity. A typical column should be 30 m long, 0.25 mm internal diameter and 0.25 μm film thickness (30 m × 0.25 mm, d_f = 0.25 mm); however, nowadays, for fast GC analysis, columns of 15 m to 30 m long and 0.10 mm to 0.15 mm internal diameter are commonly used. As the partition between carrier gas and the stationary phase is highly dependent on the temperature, the oven is usually programmed, one or more ramps, from a low temperature (*e.g.* 50 ºC) to maximum operation temperature of the column (*e.g.* 250 ºC); this procedure permit to obtain a good separation in the possible shortest time and to clean chromatographic column. Comprehensive two-dimensional gas chromatography (GC×GC) emerged recently as a powerful technique to analyse the composition of complex mixtures. GC×GC uses two columns of different characteristics, coupled in sequence through a suitable interface known as modulator, that allow peaks from the primary column to be

transferred onto the secondary column, so that an additional separation, and ideally complete resolution for all constituents, may be achieved (Weldegergis et al., 2011). The first dimension column is often non-polar and the second dimension column, shorter (1 m to 3 m), for fast analysis is polar.

The used carrier gases are nitrogen, hydrogen and helium. For a given GC system, best results, *i.e.* higher separation efficiency, are achieved with hydrogen. However, due to security factors, helium is commonly the choice. Modern equipment has electronic pneumatic controller (EPC) to maintain a constant flow during the analysis; in former systems a pressure controller is usually installed.

Detectors used commonly to analyze grape and wine volatile compounds could be classified broadly as universal or selective detectors. Flame ionization detector (FID) and mass spectrometer detector (MS) are the most used universal detectors. Atomic emission detector (AED) and electron capture detector (ECD) have been applied to halogenated compounds (Campillo et al., 2008; Schneider et al., 2003), flame photometric detector (PFD), pulsed flame photometric detector (PFPD) and sulphur chemiluminescence detector (SCD) are used in the analysis of sulphur compounds (Mestres et al., 2000). The mass spectrometer detector is the most important as it provides apart from the chromatogram an extra dimension of information, *i.e.* the mass spectra of the peak compound. Common MS routine analysis uses electron impact ionization (EI), while when searching structural information of an unknown compound, chemical ionization (CI) or even MS^n should be also applied. Quadruple (qMS) and ion trap (ITMS) analysers are the most used, either in scan mode, where scanning of all possible fragment ions within the specific range (*e.g.* 35 *m/z* to 350 *m/z*) takes place, or in the selected ion monitoring (SIM) mode, where only pre-selected ions will be detected; in the later mode, MS became a selective detector with lower detection limits. Furthermore, when faster acquisition rate is required, a time-of-flight mass analyser (TOFMS) should be used. TOFMS is fully compatible with GC×GC, providing sufficient data density for an accurate definition of the narrow peaks (Weldegergis et al., 2011).

3.2 Identification of volatile compounds

The simple way to identify volatile compounds is comparing retention times of the interest peaks with those of pure standard compounds. However, when dealing with complex mixtures, sometimes presenting non-resolved peaks, this method is very fallible; even when spiking the extract with pure standard compounds, although being safer, the identification remain problematic. For all the detectors except MS detector, this is the only way to identify volatile compounds.

When a pure standard is not commercially available and its synthesis is not possible, identification may be carried by comparing retention indexes (*e.g.* linear retention indexes, Kovats retention indexes) of compounds of interest with those of published in literature; retention indexes are based on the values attributed, by definition, to a series of homologous compounds (*e.g.* alkanes; C_8 = 800, C_9 = 900) and its relation with its retention time.

On the other hand, using a MS detector the identification is much more trustworthy because the conjugation of the fragment fingerprint of a molecule and its retention time is practically infallible. Furthermore, the conjugation of mass spectra and retention indexes may permit the identification of some suspected peaks. Mass spectra libraries are commercially available

and can be also constructed by injected and collect the spectra, under the same conditions, of purchase/synthesized pure standards; this last library gives the best results.

3.3 Quantification of volatile compounds

The quantification of the identified peaks of volatile compounds must be made after calibration of the method. External standards, internal standard and standards addition are the three approaches. Calibration with external standards involves the construction a curve, by regression of the points obtained (concentration, peak area), after the injection of several standard solutions (*e.g.* 6 points). This approach presupposes that losses occurred in samples and standard solutions treatment is exactly the same. However, samples analysis usually involve some treatment before extraction and, when injected by a micro-syringe, losses could occur during the injection; in this case one or more internal standards are added to the sample at the beginning of the procedure, in a known amount. Nevertheless, because extraction procedure and detector don't respond similarly to all volatile compounds, a relative response factor should be determined anyway. The standard additions methodology may be adequate when matrix effects occur. This method is much more time-consuming than the other two approaches because it adopts the spiking of each sample with increasing amounts of the compound to be quantified.

If the interest of an analysis is to identify a great number of compounds and/or to compare samples, it should be sufficient the use of a unique internal standard without previous determination of the response factors. The analysis is only semi-quantitative. However, when quantitative determination is imperative, the relative response factor for each compound must be determined. A more reliable quantitative analysis is conducted using labelled isotopes of each compound of interest as internal standards (Kotseridis et al., 1999).

4. GC-MS application on the evaluation of grape and wine aroma in NW Iberian Peninsula

4.1 Grapes and wines characterization

Atlantic Viticulture (NW Iberian Peninsula) include Galicia (NW Spain) and North Portugal, situated mainly on Atlantic Ocean border, where are grown several common cultivars. Five Appellations of Origin (AO) are included in Galicia, *Rías Baixas*, *Ribeiro*, *Valdeorras*, *Ribeira Sacra* and *Monterrei* and white (*Albariño, Loureira, Treixadura, Godello, Caíño blanco*) and red cultivars (*Mencia, Espadeiro, Caíño tinto, Mouratón, Brancellao, Souson*) are grown in this geographic area. In another hand, the *Vinhos Verdes* Apellation of Origin is situated in the northwest of Portugal being divided into 9 sub-regions: *Amarante, Ave, Baião, Basto, Cávado, Lima, Monção & Melgaço, Paiva* and *Sousa*. The Atlantic Ocean and the relief markedly influence the climate. There are seven recommended white grape varieties (*Alvarinho, Arinto, Avesso, Azal, Batoca, Loureiro* and *Trajadura*) and eight red grape varieties (*Amaral, Borraçal, Brancelho, Espadeiro, Padeiro de Basto, Pedral, Rabo de Ovelha* and *Vinhão*) used to produce these wines.

Albariño in Galicia (NW Spain) and *Alvarinho* in north Portugal is the most important white cultivar grown in Atlantic Viticulture, because this grape variety is only cultivated for the production of wines of recognized high quality. Contrasting to *Alvarinho* wines, malolactic fermentation is almost always applied to produce *Albariño*. Apart from *Alvarinho* and

Albariño, four other cited varieties are the same: *Espadeiro*; *Treixadura* and *Trajadura*; *Caíño tinto* and *Borraçal*; *Brancellao* and *Brancelho (*EU Project GenRes 081, 1997).

The Albariño variety, both as a grape and a wine, has been the objective of several studies (Carballeira et al., 2001; Dieguez et al. 2003; Fernández et al. 1999; Orriols & Camacho, 1991; Versini et al., 1994). Albariño from Galicia (NW Spain) was characterized by a high intensity of floral and fruity descriptors and free monoterpenes being responsible for these floral notes (Carballeira et al., 2001; Falqué et al., 2001). Ribéreau-Gayon et al. (2000) compared *Albariño* wine to *Riesling, Muscadelle* and *Sauvignon* wines and showed that *Albariño* wine was the richest in terpene compounds. Studies performed with this cultivar growed in Rias Baixas AO from Spain (Vilanova & Sieiro, 2006b) showed that the wines contained a higher concentration of terpenes in free form than in bound form. Only linalool and eugenol assumed to have the strongest odour impact on the aroma of *Albariño* wines. The *Albariño* wines were characterized by balsamic, fruity and sweet series when odour activity values (OAV) were calculated. Volatile compounds with fruity and floral odours showed the highest odour activity values, contributing in a great measure to the aroma of *Albariño* wines.

Oliveira et al. (2000) performed a comparative study on volatile composition of two aromatic cultivars from *Vinhos Verdes* AO, *Alvarinho* and *Loureiro*, characterized by freshness and floral and fruity flavours. These cultivars are employed for high quality monovarietal wine production because they are over all appreciated by their aromatic characteristics. *Loureiro*, as is already known, is an aromatic variety because of the levels of linalool in the free fraction (Oliveira et al., 2000). *Alvarinho* variety, which is poorer than *Loureiro* with respect to the free fraction, presents interesting levels of terpenic compounds in the bound fraction, as well as *Loureiro. Loureiro* and *Alvarinho* varieties have an important reserve of volatile compounds (Oliveira et al., 2000, 2004). This fact may become important in winemaking, since these compounds, particularly linalool, can be liberated from a glycoside moiety by specific enzymes and so contribute to the final wine flavour; other compounds such as monoterpenic oxides and diols, at the concentrations found in this study, may be rearranged at acidic pH to produce aromatic compounds. The ratio between (*Z*) and (*E*) isomers of 8-hydroxylinalool present in the glycosidically bound fraction seems to be important in differentiating *Alvarinho* and *Loureiro* varieties, with values near 6 for the first cultivar and about unity for the second one. The results of this work showed that it is possible to differentiate the recommended grape varieties for the *Vinhos Verdes* Region with regard to the quantification of monoterpenic compounds either in the free or in the glycosidically bound fraction. In another study, Oliveira et al. (2004) characterize five *Vinhos Verdes* grape varieties (*Alvarinho*, *Loureiro*, *Avesso*, *Amaral* and *Vinhão*) in terms of monoterpenic compounds present either in free form (17) or in glycosidically bound form (21). Nevertheless, apart from *Alvarinho* and *Loureiro*, the other three cultivars are poor respecting monoterpenic compounds.

Wines elaborated with *Loureiro* and *Alvarinho* cultivars from *Vinhos Verdes* Region showed similar composition on volatiles. *Loureiro* wines are globally richer than *Alvarinho* ones respecting monoterpenic compounds in both free and glycosidically bound forms. The varietal compounds which could influence particularly the aroma of these wines seem to be only linalool, HO-trienol, α-terpineol and β-damascenone. Terpenols seem to be more important to *Loureiro* wines and the C_{13}-norisoprenoids for *Alvarinho* ones. Respecting

fermentative compounds, *Alvarinho* wine is also particularly rich in fatty acids ethyl esters related to lipid metabolism and acetates of fusel alcohols, which can provide it a fruity character; *Loureiro* contains higher levels of esters of organic acids and 2-phenylethanol, conferring fruity and floral notes. Sensory analysis agreed with chemical analyses showing a pronounced tree and tropical fruit character for *Alvarinh*o wines while *Loureiro* wines present more intense citrus fruit notes (Oliveira et al., 2008a, 2008b). The authors indicated the possibility of discriminating *Loureiro* from *Alvarinho* wines by the ratio between (*E*) and (*Z*) isomers of 3-hexen-1-ol, in free form, and of 8-hydroxylinalool, in the glycosidically bound form.

Genisheva & Oliveira, 2009, compared the volatile composition of all white cultivars from *Vinhos Verdes* AO (*Arinto, Azal, Avesso, Batoca, Trajadura, Alvarinho* and *Loureiro*). In the free fraction, the *Loureiro* variety could be easily differentiated from the other six varieties by the important levels of linalool, above the odour perception threshold. This was in contrast to *Alvarinho, Avesso, Arinto, Azal* and *Trajadura*, where geraniol prevailed. The Batoca variety showed a very poor monoterpenic profile in the free form. *Arinto, Avesso, Azal* and *Trajadura* had a more equilibrated profile in terms of aroma compounds in both fractions. The *Arinto* variety showed a high potential of aroma compounds in the bound fraction. On the other hand, *Alvarinho* was the richest variety in respect to the glycosidically bound form, followed by *Loureiro*. In this fraction, linalool and 3,7-dimethylocta-1,5-dien-3,7-diol were the most abundant compounds in the *Loureiro* grape cultivar; additionally, the isomer (*E*)-8-hydroxylinalool was in a higher concentration than (*Z*)-8-hydroxylinalool, while for the rest of the varieties the (*Z*) isomer prevailed. The results of this study showed that it was possible to differentiate the seven recommended white varieties for the production of *Vinho Verde*, regarding free and bound monoterpene composition. Cultivars *Loureiro, Arinto* and *Alvarinho* were clearly distinct from the other studied varieties, while *Trajadura* showed an intermediate position.

Wines from minority two white cultivars (*Blanco lexítimo* and *Agudelo*) from Galicia also were characterized by Gas chromatography (Vilanova et al., 2009). These cultivars are grown in Betanzos, the most northern viticultural geographic area from Galicia (NW Spain). The results obtained suggest that ethyl octanoate (apple flavour), isoamyl acetate (banana), ethyl hexanoate (fruity) and β-damascenone (floral) were the most powerful odorants for the white wines *Blanco lexítimo* and *Agudelo* from Betanzos. *Blanco lexítimo* was the most aromatic wine dominated by citric, banana, apple and pineapple aroma. *Agudelo* wine which is a minor aromatic, compared to *Blanco lexítimo*, presents high levels of fruity aromas.

Studies about red grapes and wines from Atlantic viticulture also were performed. In recent years there has been a trend towards recovering the use of native Galician grape cultivars, whose presence had become reduced. These cultivars are well adapted to the area and transmit to their wines the characteristics of the climate and soil in which they are grown. Although currently it is produced only in small quantities, the red cultivar *Caíño* is one of the most appreciated of the *Rías Baixas* and *Ribeiro* Appellation of Origin areas from NW Spain. *Caíño tinto, Caíño longo* and *Caíño bravo* were analysed by gas chromatography (Vilanova et al., 2007a). The wines made from the different cultivars and the vintages were clearly different. *Caíño longo* wines had the highest concentrations of acetates and esters. The concentrations of ethyl esters and acetates in *Caíño bravo* wines were comparatively very low. From an oenological point of view, the *Caíño tinto* was the most interesting wine

because its composition was the most equilibrated. Non-terpenic compounds were the most abundant aroma substances in the considered varieties (Vilanova et al., 2008).

Wines produced from other minority *Vitis vinifera* red cultivars *Castañal* and *Serradelo* from Galicia (NW Spain) also were studied (Vilanova & Martinez 2007; Vilanova et al., 2009). From the 36 compounds identified in *Castañal* wine, 10 were determined as the most powerful odorants: β-ionone, 3-methyl-1-butanol, benzyl alcohol, 2-phenylethanol, ethyl acetate, isoamyl acetate, ethyl lactate, ethyl butyrate, ethyl hexanoate and ethyl octanoate. These data suggested *Castañal* wines as a fruity (blackberry) and floral (rose) product (Vilanova & Martinez, 2007). In another hand, ethyl octanoate and β-damascenone (fruity and floral aroma, respectively) were the most odorant for the red wine *Serradelo* form Betanzos (Vilanova et al., 2009). Ethyl octanoate and β-damascenone were the most odorant for the *Serradelo* red wine.

4.2 *Terroir* effect on grape and wine volatile composition

Terroir has been acknowledged as an important factor in grape and wine quality, particularly in European viticulture. The *terroir* concept was born in the Europe Appellations of Origin (AO) and was used for many purposes: to guarantee the authenticity of the products against frauds, to justify an economical advantage linked to a specific property, to synthesize an historical local experience, to strengthen the defence of a community of growers facing economical competition and to explain the characteristics of the wines.

Quite recently, around the 1980′s, a scientific approach of *terroir* was developed by several teams and led to establish some relations between some elements of the natural environment and the grape. Today *terroir* concept can be defined as interactive ecosystem, including climate, soil and the vine (van Leeuwen et al., 2004).

The Atlantic Ocean and the relief markedly influence the climate. This region is in Viticultural Zone C I a) of the Winegrowing Regions of the European Communities. The produced wine is unique and its specific characteristics are mainly due to the climate and soil. Several studies have been conducted to know the grape and wine volatile composition in basis to the *terroir* in Atlantic viticulture (Oliveira et al., 2000; Vilanova et al., 2007b; Zamuz & Vilanova, 2006a, 2006b).

The white *Vinho Verde* is softly alcoholic, with a delicate, fresh and fruity bouquet. Oliveira et al., (2000) investigated the influence of the climate and the soil on the volatile compounds of grapes from two autochthonous white grape varieties, *Alvarinho* and *Loureiro*, in two sub-regions for each one of them (*Alvarinho* in *Monção* and *Lima*; *Loureiro* in *Lima* and *Cávado*). The results showed that the global characteristics of the grapes from the two varieties depend on the harvest factor (climate) rather than on the sub-region where the vine is planted. The characteristics of the soil have an even lesser influence on the variableness of the samples, although the *Alvarinho* variety picked in the *Lima* sub-region seems to have different characteristics.

Albariño must and wine from NW Spain was studied in three different areas from *Rías Baixas* AO (Vilanova et al., 2007b; Zamuz & Vilanova, 2006a). The Rías Baixas was originally made up of three subzones, *Val do Salnés*, *O Rosal* and *Condado do Tea*. The Atlantic climate, with

wet winters and sea mists, varies between the subzones. The coolest is *Val do Salnés*, and the hottest is southerly *Condado do Tea* and *O Rosal*, with occasional temperatures over 35 °C and colder winters. Differences of climate and geography make the wines from the different origins individual in their own right. Non-terpenic compounds were the most abundant compounds in the free aroma fraction of the *Albariño* musts from the three geographic areas; of these, 2-phenylethanol (rose aroma) was the most important. The *Albariño* must from *O Rosal* should be the most aromatic since it had significantly higher volatile compounds content, with the bound compounds making up the largest group, quantitatively. The *Albariño* must from *Val do Salnés* had the lowest concentrations of volatile compounds and should therefore be the least aromatic; this could be related to its lower maturation index.

Respecting wines, *Albariño* from *O Rosal* was characterized by a high content of higher alcohols, while wines from *Val do Salnés* show the highest concentrations of free terpenes, acetates and ethyl esters. Wines of *Condado do Tea* show the highest concentrations of C_{13}-norisoprenids, principally due to α-ionone (Zamuz & Vilanova, 2006b). The results obtained in the study of *Albariño* wines showed that significant differences have been found among different geographic areas of *Rías Baixas* AO (northwestern Spain) in terms of the concentrations of most aromatic compounds.

Another comparative study was conducted on *Albariño* wine produced from musts from northern and southern Galicia (NW Spain) (Vilanova et al., 2007b). The influence of *terroir* on varietal and fermentative volatile compounds was studied. Data obtained from gas chromatography showed that differences were present in wine volatiles. The *Albariño* wines from northern Galicia showed the highest total concentration of volatiles analysed, dominated by higher contents in total free terpenes and acetates. Total higher alcohols and ethyl esters characterised the *Albariño* wine from the south. Among the terpenes found, geraniol was markedly abundant in the north, while nerol and linalool were most abundant in the south. Among the alcohols, 2-phenylethanol and benzyl alcohol showed the highest concentrations in the south and in the north, respectively. *Albariño* wines from the south were more heterogenic than those from the north. Differentiation of these wines was possible. This behaviour could be due to the predominance of terroir over the varietal character of the wines.

4.3 Vinification techniques influence on volatile composition of wines

Changes in volatiles during maturation in bottles of monovarietal *Vinhos Verdes* wines from *Loureiro* and *Alvarinho* grape varieties were followed by chemical and sensory analyses (Oliveira et al., 2008a). Young wines and wines matured for 8 and 20 months were studied. The volatiles were determined by gas chromatography-mass spectrometry (GC-MS) after extraction on XAD-2 resin. Straight chain fatty acid ethyl esters and acetates of fusel alcohols decreased quicker for *Loureiro* wine, while the increase in ethyl esters of branched fatty acids was similar for both varieties. Linalool, HO-trienol, α-terpineol and β-damascenone could be used to differentiate between each variety. However, linalool decreased to negligible values after 20 months of maturation. β-damascenone decreased but remained high enough to be useful for differentiating each variety. Sensory analysis indicated a decrease of tropical fruit and tree fruit characters with conservation time for *Alvarinho* wine, and the opposite for *Loureiro;* moreover, citrus fruit character decreased in both varieties.

4.4 Yeast influence on volatile composition of wines

The yeast responsible for alcoholic fermentation in winemaking is usually introduced into the must from the surface of the grapes, the surface of winery equipment, or from specifically prepared cultures. The fermentation process can occur either naturally, without inoculation, or by inoculating the must with selected starters. The use of locally selected yeast strains (usually belonging to the species *Saccharomyces cerevisiae*), with strain-specific metabolic characteristics can positively affect the final quality of the wine (Regodon et al., 1997; Romano et al., 2003). Several studies have clearly shown the effects of indigenous and inoculated yeast populations on the wine volatile composition (Mateo et al., 2001; Nurgel et al., 2003).

From Atlantic viticulture, a comparative study was made of the fermentation products of Spanish *Albariño* wines produced with spontaneous yeast flora and an indigenous selected *Saccharomyces cerevisiae* strain (Vilanova & Sieiro, 2006a). The gas chromatography data showed that the wines differed in their volatiles contents. The wine produced by spontaneous fermentation showed higher contents in higher alcohols, ethyl esters (except ethyl hexanoate and ethyl octanoate) and acetates. Alb16 selected yeast strain led to the production of extra ethyl hexanoate and hexyl acetate. The wines obtained by the spontaneous fermentation were more aromatic than those obtained with the Alb16 yeast strain.

Another study (Vilanova & Masneuf-Pomarède, 2005a, 2005b) was performed with *S. cerevisiae* cerevisiae yeast strains (ASln1, ASln2 and ASln20) isolated from musts obtained from *Albariño* grapes harvested in the *Rías Baixas* region (Spain). ASln20 produced the highest amounts of alcohol. Marked differences in the volatile composition of the fermented musts, determined by GC were dependent upon the yeast strain used. *S. cerevisiae* ASln1 and ASln20 produced the greatest quantities of esters, (ethyl butyrate, ethyl hexanoate, ethyl octanoate and ethyl decanoate) which can give to wine a fruity aroma. These results suggest that the production level of these compounds is characteristic of the individual yeast strains, which highlights the importance of characterising yeast strains for industrial use.

5. Correlation between volatiles by GC and sensory properties of wines

To understand the chemical compounds in wine that shown sensory characteristics, is necessary some information regarding both, the volatile composition and the sensory properties. Gas chromatography is an important analysis technique of wine volatile components, although the aromatic impact of volatiles identified is evaluated, generally by determining perception thresholds.

The odour activity value (OAV) is a useful parameter to assess the relative importance of individual chemical components present in a wine. The aroma active compounds are volatiles whose concentration in wine is above their odour threshold (OAV>1). However, even when the OAV of a particular compound is less than 1, it still might contribute to the aroma of a wine as a consequence of some additive or synergic effect among compounds with similar aroma nature.

In another hand, sensory analysis invoices the detection and description of qualitative and quantitative sensory components of a product by trained panel of judges (Meilgaard et al.,

1999). Quantitative Descriptive Analysis (Stone & Sidel, 1998) is one of the most comprehensive and informative tools used in sensory analysis. This technique can provide complete sensory descriptions of a product as wine.

Relationships between volatile composition and sensory descriptors of wines have been explored by other researchers (Barbe et al., 2008; Francis & Newton, 2005). Several authors have suggested the use of multivariate strategies such as Partial Least Square (PLS) regression to predict sensory descriptors from chemical composition in wine (Koussissi & Paterson, 2007; Tenenhaus et al., 2005).

The study of correlation between volatile compounds by instrumental analysis and sensory properties of *Albariño* wine from NW Spain was performed by Vilanova et al. (2010). The results of the investigation showed the compounds that most contributed to the flavour of *Albariño* wines in instrumental analysis were those related to fruity (ethyl esters and acetates) and floral aromas (monoterpenes). Similar results were found in sensory analysis where the descriptors with the highest Geometric Mean were fruity and floral aromas too (citric, flowers, fruit, ripe fruit, apple and tropical). Therefore, this work demonstrates that some relationship between sensory data and volatile compounds exist to asses sensory properties in *Albariño* wines.

Other studies analysed the aroma of wines from several minority red cultivars from NW Spain by Gas Chromatography Mass Spectrometry (GC-MS) and sensory descriptive analysis (SDA) (Canosa et al. 2011; Vilanova et al., data not published). Sixteen volatile compounds and twelve sensory attributes showed significant differences among red wines *Brancellao, Mencía, Merenzao, Mouratón* and *Sousón*. Twenty out of fifty one quantified volatile compounds were present in some samples at concentrations higher than their corresponding odour thresholds (*OAV* >1), thus contributing to the final wine aroma. Principal component analysis (PCA) applied to volatile compounds showed three groups of cultivars: *Mencía-Brancellao, Mouratón-Merenzao* and *Sousón. Souson* and *Mouraton* wines were the most different from a sensory viewpoint, both cultivars being clearly linked to valuable traits like aroma and taste intensity and quality. Partial Least Square (PLS) regression applied to volatile compounds and aroma descriptors yielded a satisfactory model for the prediction of four important aroma descriptors in this set of wines - aroma quality, aroma intensity, herbaceous and red fruit - from instrumental analysis data. Free and bound compounds from *Pedral* and *Espadeiro* red cultivars (NW Spain) also were studied (Canosa et al., 2011). *Pedral* cultivar showed an important contribution of glycosidically bound compounds, especially C_{13}-norisoprenoids.

6. References

Abdel-Rehim, M. (2010). Recent advances in microextraction by packed sorbent for bioanalysis. *Journal of Chromatography A* 1217, 2569–2580

Allen, M.S., Lacey M.J. & Boyd S. (1994) Determination of methoxypyrazines in red wines by stable isotope dilution gas chromatography-mass spectroscopy. *Journal of Agriculture and Food Chemistry* 42, 1734–1738

Allen, M.S.; Lacey, M.J.; Harris, R.L.N. & Brown, W.V. (1991). Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *American Journal of Enology and Viticulture* 42, 109-112

Altun, Z.; Abdel-Rehim, M. & Blomberg, L.G. (2004). New trends in sample preparation: on-line microextraction in packed syringe (MEPS) for LC and GC applications Part III: Determination and validation of local anaesthetics in human plasma samples using a cation-exchange sorbent, and MEPS–LC–MS–MS. *Journal of Chromatography B* 813, 129–135

Andujar-Ortiz, I.; Moreno-Arribas, M.V.; Martín-Álvarez, P.J. & Pozo-Bayón M.A. (2009). Analytical performance of three commonly used extraction methods for the gas chromatography–mass spectrometry analysis of wine volatile compounds. *Journal of Chromatography A* 1216, 7351–7357

Arthur, C.L. & Pawliszyn, J. (1990). Solid phase microextraction with thermal desorption using fused silica optical fibers. *Analytical Chemistry* 62, 2145–2148

Asensio-Ramos, M.; Ravelo-Pérez, L.M.; González-Curbelo, M.A. & Hernández-Borges, J. (2011). Liquid phase microextraction applications in food analysis. *Journal of Chromatography A* 1218, 7415–7437

Baltussen, E.; Sandra, P.; David, F. & Cramers, C. (1999). Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and Principles. *Journal of Microcolumn Separations* 11 (10), 737–747

Barbe, J.C.; Pineau, B. & Silva, A.C. (2008). Instrumental and sensory approaches for the characterization of compounds responsible for wine aroma. *Chemistry and Biodiversity* 5, 1170–1183.

Baumes, R.L.; Cordonnier, R.E.; Nitz, S. & Drawert, F. (1986) Identification and determination of volatile constituents in wines from different vine cultivars. *Journal of the Science of Food and Agriculture* 37, 927–943

Bayonove, C.L.; Baumes, R.L.; Crouzet, J. & Günata, Y.Z. (1998). Arômes. *In: Œnologie - Fondements Scientifiques et Technologiques*, Chap. nº 5, Lavoisier Tec & Doc (Eds.), Paris, 163–235

Bell, A.A.; Ough, C.S. & Kliewer, W.M. (1979). Effects on must and wine composition, rates of fermentation, and wine quality of nitrogen fertilization of *Vitis vinifera* var. Thompson Seedless grapevines. *American Journal of Enology and Viticulture* 30 (2), 124–129

Blanch, G.P.; Reglero, G. & Herraiz, M. (1995). Analysis of wine aroma by off-Line and on-line supercritical fluid extraction-gas chromatography. *Journal of Agriculture and Food Chemistry* 43, 1251–1258

Blanch, G.P.; Reglero, G. & Herraiz, M. (1996). Rapid extraction of wine aroma compounds using a new simultaneous distillation-solvent extraction device. *Food Chemistry* 56 (4), 439–444

Bohlscheid, J.C.; Wang, X.-D.; Mattinson, S.D. & Edwards, C.G. (2006). Comparison of headspace solid phase microextraction and XAD-2 methods to extract volatile compounds by *Saccharomyces cerevisiae* during wine fermentations. *Journal of Food Quality* 29, 1–15

Bonnländer, B.; Baderschneider, B.; Messerer, M. & Winterhalter, P. (1998). Isolation of two novel terpenoid glucose esters from Riesling wine. *Journal of Agriculture and Food Chemistry* 46 (4), 1474–1478

Cabaroglu, T.; Canbas, A.; Baumes, R.L.; Bayonove, C.L.; Lepoutre, J.P. & Günata, Y.Z. (1997). Aroma composition of a white wine of *Vitis vinifera* L. cv. Emir as affected by skin contact. *Journal of Food Science*, 62 (4), 680–683

Cabredo-Pinillos, S.; Cedrón-Fernández, T.; González-Briongos, M.; Puente-Pascual, L. & Sáenz-Barrio, C. (2006). Ultrasound-assisted extraction of volatile compounds from wine samples: Optimisation of the method. *Talanta* 69, 1123–1129

Campillo, N.; Peñalver, R. & Hernández-Córdoba, M. (2008). Solid-phase microextraction for the determination of haloanisoles in wines and other alcoholic beverages using gas chromatography and atomic emission detection. *Journal of Chromatography A* 1210, 222–228

Campillo, N.; Vinas, P.; Cacho, J.I.; Peñalver, R. & Hernández-Córdoba, M. (2010). Evaluation of dispersive liquid–liquid microextraction for the simultaneous determination of chlorophenols and haloanisoles in wines and cork stoppers using gas chromatography–mass spectrometry. *Journal of Chromatography A* 1217, 7323–7330

Canosa, P.; Oliveira, J.M.; Masa, A. & Vilanova, M. (2011). Study of the volatile and glycosidically bound compounds of minority *Vitis vinifera* red cultivars from NW Spain. *Journal of the Institute of Brewing* 117(3), 462–471

Carballeira, L.; Cortés, S.; Gil, M.L. & Fernández, E. (2001). Determination of aromatic compounds, during ripening, in two white grape varietes, by SPE-GC. *Chromatographia* 53(suppl), S350–S355

Carro, N.; García, C.M. & Cela, R. (1997). Microwave-assisted extraction of monoterpenols in must samples. *Analyst* 122, 325–329

Castro, R.; Natera, R.; Durán, E. & García-Barroso, C. (2008). Application of solid phase extraction techniques to analyse volatile compounds in wines and other enological products. *European Food Research and Technology* 228, 1–18

Caven-Quantrill, D.J. & Buglass, A.J. (2006). Comparison of micro-scale simultaneous distillation–extraction and stir bar sorptive extraction for the determination of volatile organic constituents of grape juice. *Journal of Chromatography A* 1117, 121–131

Cocito, C.; Gaetano, G. & Delfini, C. (1995). Rapid extraction of aroma compounds in must and wine by means of ultrasound. *Food Chemistry* 52, 311–320

Coelho, E.; Coimbra, M.A.; Nogueira, J.F. & Rocha, S.M. (2009) Quantification approach for assessment of sparkling wine volatiles from different soils, ripening stages, and varieties by stir bar sorptive extraction with liquid desorption. *Analytica Chimica Acta* 635, 214–221

Cortada, C.; Vidal, L. & Canals, A. (2011). Determination of geosmin and 2-methylisoborneol in water and wine samples by ultrasound-assisted dispersive liquid–liquid microextraction coupled to gas chromatography–mass spectrometry. *Journal of Chromatography A* 1218, 17–22

Crouzet, J.; Flanzy, C.; Günata, Y.Z.; Pellerin, P. & Sapis, J.C. (1998). Les enzymes en œnologie. *In: Œnologie - Fondements Scientifiques et Technologiques*, Chap. nº 10, Lavoisier Tec & Doc (Eds.), Paris, 361–411

Culleré, L.; Aznar, M.; Cacho, J. & Ferreira, V. (2003). Fast fractionation of complex organic extracts by normal-phase chromatography on a solid-phase extraction polymeric sorbent. Optimization of a method to fractionate wine flavor extracts. *Journal of Chromatography A* 1017, 17–26

Czerny, M.; Christlbauer, M.; Christlbauer, M.; Fischer, A.; Granvogl, M.; Hammer, M.; Hartl, C.; Hernandez, N.M. & Schieberle P. (2008). Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *European Food Research and Technology* 228, 265–273

Darriet, P.; Lavigne-Cruège, V. & Tominaga, T. (1999). Un paradoxe: les composés soufrés volatils responsables de défauts et de qualités dans les vins. *In: La Dégustation. Journal International des Sciences de laVigne et du Vin nº hors série*, 137–143

David, D. & Sandra, P. (2007). Stir bar sorptive extraction for trace analysis. *Journal of Chromatography A* 1152, 54–69

Di Stefano, R. & Castino, M. (1983). Evoluzione dei composti di natura terpenica durante la conservazione dell'Asti spumante. *Rivista di Viticoltura e di Enologia di Conegliano* 5, 245-258

Díaz-Maroto, M.C.; Schneider, R. & Baumes, R. (2005). Formation pathways of ethyl esters of branched short-chain fatty acids during wine aging. *Journal of Agriculture and Food Chemistry* 53 (9), 3503–3509

Dieguez, S.C.; Lois, L.C.; Gómez, E.F. & de la Peña M.L.G. (2003). Aromatic composition of the *Vitis vinifera* grape Albariño. *LWT-Food Science and Technology* 36, 585–590

Díez, J.; Domínguez, C.; Guillén, D.A.; Veas, R. & Barroso, C.G. (2004). Optimisation of stir bar sorptive extraction for the analysis of volatile phenols in wines. *Journal of Chromatography A* 1025, 263–267

D'Incecco, N.; Bartowsky, E.; Kassara, S.; Lante, A.; Spettoli, P. & Henschke, P.A. (2004). Release of glycosidically bound flavour compounds of Chardonnay by *Oenococcus oeni* during malolactic fermentation. *Food Microbiology* 21, 257–265

Dubois, P. (1993). Les arômes des vins et leurs defauts. *Revue Française d'Œnologie* 144, 63–72

Dubois, P. (1994). Les arômes des vins et leurs defauts (cont.). *Revue Française d'Œnologie* 145, 27–40

Dugelay, I. (1993). L'arôme du raisin: étude des précurseurs hétérosidiques et des activités enzymatiques exogénes impliquées dans leur hydrolyse. Applications technologiques. *Thèse Doctorat*, École Supérieure Agronomique de Montpellier

Dupin, I.; Günata, Y.Z.; Sapis, J.C. & Bayonove, C.L. (1992). Prodution of β-apiosidase by *Aspergillus niger*. Partial purification, properties, and effect on terpenyl apiosilglucosides from grape. *Journal of Agriculture and Food Chemistry* 40 (10), 1886–1891

Enzell, C. (1985). Biodegradation of carotenoids - an important route to aroma compounds. *Pure Applied Chemistry* 57 (5), 693–700

Escudero, A.; Gogorza, B.; Melus, M.A.; Ortin, N.; Cacho, J. & Ferreira, V. (2004). Characterization of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *Journal of Agriculture and Food Chemistry* 52 (11), 3516–3524

Etiévant, P.X. (1991). Wine. *In: Volatile Compounds in Foods and Beverages*, Chap. nº 14, Henk Maarse (Eds.), Marcel Dekker Inc., New York, pp. 483–546

Etiévant, P.X.; Issanchou, S.N. & Bayonove, C.L. (1983). The flavour of Muscat wine: The sensory contribution of some volatile compounds. *Journal of the Science of Food and Agriculture* 34, 497–504

EU Project GenRes 081. (1997). Primary Descriptor List for Grapevine Cultivars and Species (*Vitis* L.). Institut für Rebenzüchtung Geilweilerhof, Siebeldingen, Germany.

Falqué, E.; Fernández, E. & Dubourdieu, D. (2001). Differentiation of white wines by their aromatic index. *Talanta* 45, 271–281

Fang, Y. & Qian, M.C. (2006). Quantification of selected aroma-active compounds in Pinot noir wines from different grape maturities. *Journal of Agricultural and Food Chemistry* 54, 8567–8573

Fernández, E.; Cortés, S.M.; Castro, M.; Gil, M. & Gil, M.L. (1999). Distribution of free and glycosidically bound monoterpenes and norisoprenoids in the skin and pulp ofAlbariño grapes during 1998 maturation. In: *Oenologie 99* – 6th Symposium International dOenologie, A. Lonvaud Funel (ed.), Bordeaux. TEC&DOC: Paris, pp. 161–164

Ferreira, V. & Cacho, J. (2009). Identification of Impact odorants of wines. In: *Wine Chemistry and Biochemistry*, MV. Moreno-Arribas and M.C. Polo (eds.). Springer

Ferreira, V.; Lopez, R. & Cacho, J.F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture* 80 (11), 1659–1667

Francis, I.L. & Newton, J.L. (2005). Determining wine aroma from compositional data. *Australian Journal of Grape and Wine Research* 11, 114–126

Genisheva, Z. & Oliveira, J.M. (2009). Monoterpenic characterization of white cultivars from Vinhos Verdes appellation of origin. (North Portugal) *Journal of the Institute of Brewing* 115(4), 308–317

Giraudel, J.L.; Setkova, L.; Pawliszyn, J &, Montury, M. (2007). Rapid headspace solid-phase microextraction–gas chromatographic–time-of-flight mass spectrometric method for qualitative profiling of ice wine volatile fraction III. Relative characterization of Canadian and Czech ice wines using self-organizing maps. *Journal of Chromatography A* 1147, 241–253

Grob, R.L. & Barry, E.F. (2004). *Modern practice of chromatography*. 4th Edition, John Wiley & Sons Inc., ISBN 0-471-22983-0, Hoboken, New Jersey

Günata, Y.Z.; Bayonove, C.L.; Baumes, R.L. & Cordonnier, R.E. (1985). The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some aroma components. *Journal of Chromatography A* 331, 83–90

Günata, Y.Z.; Bitteur, S.M.; Brillouet, J.M.; Bayonove, C.L. & Cordonnier, R.E. (1988). Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. *Carbohydrate Research*, 184, 139–149

Günata, Y.Z.; Dugelay, I.; Sapis, J.C.; Baumes, R.L. & Bayonove, C.L. (1993). Role of enzymes in the use of the flavour potential from grape glycosides in winemaking. *In: Progress in Flavour Precursor Studies-Analysis, Generation, Biotechnology*, P. Schreier and P. Winterhalter (Eds.). Allured Pub., Carol Stream, pp. 219–234

Guth, H. (1996). Determination of the configuration of wine lactone. *Helvetica Chimica Acta* 79, 1559–1571

Guth, H. (1997). Quantitation and sensory studies of character impact odorants of different white wine varieties. *Journal of Agiculture and Food Chemistry* 45, 3027–3032

Henschke, P. & Jiranek, V. (1993) Yeasts–metabolism of nitrogen compounds. In: *Wine. Microbiology and biotechnology, G.H.* Fleet (Ed), Harwood Academic, Chur, pp. 77–164

Hernanz, D.; Gallo, V.; Recamales, Á.F., Meléndez-Martínez, A.J. & Heredia, F.J. (2008). Comparison of the effectiveness of solid-phase and ultrasound-mediated liquid–liquid extractions to determine the volatile compounds of wine. *Talanta* 76, 929–935

Ho, T.D.; Canestraro, A.J. & Anderson, J.L. (2011). Ionic liquids in solid-phase microextraction: A review. *Analytica Chimica Acta* 695, 18–43

Ibarz, M.J.; Ferreira, V.; Hernández-Orte, P.; Loscos, N. & Cacho, J. (2006). Optimization and evaluation of a procedure for the gas chromatographic–mass spectrometric analysis of the aromas generated by fast acid hydrolysis of flavor precursors extracted from grapes. *Journal of Chromatography A* 1116, 217–229

Jeannot, M.A. & Cantwell, F.F. (1997), Mass transfer characteristics of solvent extraction into a single drop at the tip of a syringe needle. *Analytical Chemistry* 69, 235–239

Jofré, V.P.; Assof, M.V.; Fanzone, M.L.; Goicoechea, H.C.; Martínez, L.D. & Silva, M.F. (2010). Optimization of ultrasound assisted-emulsification-dispersive liquid–liquid microextraction by experimental design methodologies for the determination of

sulfur compounds in wines by gas chromatography–mass spectrometry. *Analytica Chimica Acta* 683, 126–135

Jönsson, S.; Hagberg, J. & van Bavel, B. (2008). Determination of 2,4,6-Trichloroanisole and 2,4,6-Tribromoanisole in Wine Using Microextraction in Packed Syringe and Gas Chromatography-Mass Spectrometry. *Journal of Agricultural and Food Chemistry* 56, 4962–4967

Karagiannis, S.; Economou, A.; Lanaridis, P. (2000) Phenolic and volatile composition of wines Made from *Vitis vinifera* Cv. Muscat lefko grapes from the island of Samos. *Journal of Agriculture and Food Chemistry 48*, 5369–5375

Karásek, P.; Planeta, J.; Ostrá, E.V.; Mikesová, M.; Goliás, J.; Roth, M. & Vejrosta, J. (2003) Direct continuous supercritical fluid extraction as a novel method of wine analysis. Comparison with conventional indirect extraction and implications for wine variety identification. *Journal of Chromatography A* 1002, 13–23

Kataoka, H.; Lord, H.L. & Pawliszyn, J. (2000). Applications of solid-phase microextraction in food analysis. *Journal of Chromatography A* 880, 35–62

Kotseridis, Y., Baumes, R.L.; Bertrand, A. & Skouroumounis, G.K. (1999). Quantitative determination of β-ionone in red wines and grapes of Bordeaux using a stable isotope dilution assay. *Journal of Chromatography A* 848, 317–325

Koussissi, E.,A. & Paterson, J.R. (2007). Piggott, Sensory profiling of aroma in Greek dry red wines using rank-rating and monadic scoring related to headspace composition. *European Food Research and Technology* 225, 749–756

Lacey, M.J.; Allen, M.S.; Harris, R.L.N. & Brown, W.V. (1991). Methoxypyrazines in Sauvignon Blanc grapes and wines. *American Journal of Enolology and Viticultute* 42 (2), 103–108

Liu, H. & Dasgupta, P.K, (1996). Analytical chemistry in a drop. Solvent extraction in a microdrop. *Analytical Chemistry* 68, 1817–1821

Liu, M.; Zeng, Z. & Tian, Y. (2005). Elimination of matrix effects for headspace solid-phase microextraction of important volatile compounds in red wine using a novel coating. *Analytica Chimica Acta* 540, 341–353

López, R.; Aznar, M.; Cacho, J. & Ferreira, V. (2002). Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *Journal of Chromatography A* 966, 167–177

López, R.; Gracia-Moreno, E.; Cacho, J. & Ferrreira, V. (2011). Development of a mixed-mode solid phase extraction method and further gas chromatography mass spectrometry for the analysis of 3-alkyl-2-methoxypyrazines in wine. *Journal of Chromatography A* 1218, 842–848

Luan, F.; Mosandl, A.; Gubesch, M. & Wüst M. (2006). Enantioselective analysis of monoterpenes in different grape varieties during berry ripening using stir bar sorptive extraction- and solid phase extraction-enantioselective-multidimensional gas chromatography-mass spectrometry. *Journal of Chromatography A* 1112, 369–374

Luan, F.; Mosandl, A.; Münch, A. & Wüst, M. (2005). Metabolism of geraniol in grape berry mesocarp of *Vitis vinifera* L. cv. Scheurebe: demonstration of stereoselective reduction, *E/Z*-isomerization, oxidation and glycosylation. *Phytochemistry* 66, 295–303

Marais, J. & Pool, H.J. (1980) Effect of storage time and temperature on the volatile composition and quality of dry white table wines. *Vitis* 19, 151–164

Marais, J.; Versini, G.; van Wyk, C.J. & Rapp, A. (1992). Effect of region on free and bound monoterpene and C_{13} norisoprenoid concentration in Weisser Riesling wines. *South African Journal of Enology and Viticulture* 13, 71–77

Marín, J.; Zalacain, A.; de Miguel, C.; Alonso, G.L. & Salinas, M.R. (2005). Stir bar sorptive extraction for the determination of volatile compounds in oak-aged wines. *Journal of Chromatography A* 1098, 1–6

Márquez-Sillero, I.; Aguilera-Herrador, E.; Cárdenas, S. & Valcárcel, M. (2011). Determination of 2,4,6-tricholoroanisole in water and wine samples by ionic liquid-based single-drop microextraction and ion mobility spectrometry. *Analytica Chimica Acta* 702, 199–204

Martendal, E.; Budziak, D. & Carasek, E. (2007). Application of fractional factorial experimental and Box-Behnken designs for optimization of single-drop microextraction of 2,4,6-trichloroanisole and 2,4,6-tribromoanisole from wine samples. *Journal of Chromatography A* 1148, 131–136

Mateo, J.J.; Jimenez, M.; Pastor, A. & Huerta, T. (2001). Yeast starter cultures affecting wine fermenting and volatiles. *Food Research International* 34, 307–314

Mateo-Vivaracho, L.; Cacho, J. & Ferreira, V. (2009). Selective preconcentration of volatile mercaptans in small SPE cartridges: Quantitative determination of trace odor-active polyfunctional mercaptans in wine. *Journal of Separation Science* 32, 3845–3853

Meilgaard, M.C. (1975). Flavor chemistry of beer: Part II: Flavor and threshold of 239 aroma volatiles. *MBAA Technical Quarterly* 12 (3), 151–168

Meilgaard, M.C.; Civille, G.V. & Carr B.T. (1999). *Sensory Evaluation Techniques*. CRC Press, 3rd edition, ISBN: 978-0-8493-0276-3, Boca Raton, United States of America

Mestres, M.; Busto, O. & Guasch, J. (2000). Analysis of organic sulfur compounds in wine aroma. *Journal of Chromatography A* 881, 569–581

Mestres, M.; Sala, C.; Martí, M.P.; Busto, O. & Guasch, J. (1999). Headspace solid-phase microextraction of sulphides and disulphides using Carboxen–polydimethylsiloxane fibers in the analysis of wine aroma. *Journal of Chromatography A* 835, 137–144

Moio, L.; Ugliano, M., Genovese, A.; Gambuti, A.; Pessina, R. & Piombino, P. (2004). Effect of antioxidant protection of must on volatile compounds and aroma shelf life of Falanghina (*Vitis vinifera* L.) wine. *Journal of Agriculture and Food Chemistry* 52 (4) 891–897

Morales-Valle, H.; Silva, L.C.; Paterson, R.M.; Oliveira, J.M.; Venâncio, A. & Lima, N. (2010). Microextraction and gas chromatography/mass spectrometry for improved analysis of geosmin and other fungal "off" volatiles in grape juice. *Journal of Microbiological Methods* 83, 48–52

Moreno, J.A.; Zea, L.; Moyano, L. & Medina, M. (2005) Aroma compounds as markers of the changes in sherry wines subjected to biological ageing. *Food Control* 16, 333-338

Morrot, G. & Brochet, F. (2000) Ce que le nez peut dire. In: *La Dégustation. Journal International des Sciences de la Vigne et du Vin, nº Hors Série*, 15–18

Nicolini, G.; Versini, G. & Amadei, E. (1996). Caratteristiche qualitative del vino Müller-Thurgau del Trentino in relazione ad interventi di tecnica enologica. *Rivista Viticulture Enology* 49 (2), 37–57

Nurgel, C.; Erten, H.; Canbas, A.; Cabaroglu, T.& Selli, S. (2003). Fermentative aroma in wines from *Vitis vinifera* cv. Kalecik karasi in relation with inoculation with selected dry yeasts. *Journal International des Sciences de la Vigne et du Vin* 37, 155–161

Nykänen, L. (1986). Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *American Journal of Enology and Viticulture* 37 (1), 84–96

Oliveira, J.M.; Araújo, I.M.; Pereira, O.M.; Maia, J.S.; Amaral, A.J. & Maia, M.O. (2004). Characterization and differentiation of five Vinhos Verdes grape varieties on the basis of monoterpenic compounds. *Analytica Chimica Acta* 513, 269–275

Oliveira, J.M.; Faria, M.; Sá, F.; Barros, F. & Araújo, I.M. (2006). C_6-alcohols as varietal markers for assessment of wine origin. *Analytica Chimica Acta* 563, 300–309

Oliveira, J.M.; Maia, M.O.; Baumes, R.L. & Bayonove, C.L. (2000). Free and bound aromatic components of *Loureiro* and *Alvarinho* grape varieties from the *Vinhos Verdes* region. *Viticultural and Enological Sciences* 55, 13–20

Oliveira, J.M.; Oliveira, P.; Baumes, R.L. & Maia, M.O. (2008a). Changes in aromatic characteristics of *Loureiro* and *Alvarinho* wines during maturation. *Journal of Food Composition and Analysis* 21, 695–707

Oliveira, J.M.; Oliveira, P.; Baumes, R.L. & Maia, M.O. (2008b). Volatile and glycosidically bound composition of Loureiro and Alvarinho wines. *Food Science and Technology International* 14 (4), 341–353

Ong, P.C. & Acree, T.E. (1999). Similarities in the aroma chemistry of Gewürztraminer variety wines and lychee (*Litchi chinesis* Sonn.) fruit. *Journal of Agriculture and Food Chemistry* 47(2), 665–670

Orriols, I. & Camacho, F.M. (1991). Influencia de las levaduras en la formación de sustancias volátiles en la vinificación de la variedad Albariño. *Vitivinicultura* 2, 21–24

Ortega, C.; López, R.; Cacho, J.; Ferreira, V. (2001). Fast analysis of important wine volatile compounds. Development and validation of a new method based on gas chromatographic–flame ionisation detection analysis of dichloromethane microextracts. *Journal of Chromatography A* 923, 205–214

Palma, M.; Taylor, L.T; Zoecklein, B.W. & Douglas, L.S. (2000). Supercritical fluid extraction of grape glycosides. *Journal of Agriculture and Food Chemistry* 48, 775–779

Pawliszyn, J. (2009). Handbook of solid phase microextraction, *Chemical Industry Press of China,* ISBN-13: 978-7-122-04701-4

Peña, R.M.; Barciela, J.; Herrero, C. & García-Martín, S. (2005). Comparison of ultrasound-assisted extraction and direct immersion solid-phase microextraction methods for the analysis of monoterpenoids in wine. *Talanta* 67, 129–135

Perestrelo, R.; Fernandes, A.; Albuquerque, F.F.; Marques, J.C. & Câmara, J.S. (2006). Analytical characterization of the aroma of Tinta Negra Mole red wine: Identification of the main odorants compounds. *Analytica Chimica Acta* 563, 154–164

Pizarro, C.; Pérez-del-Notario, N. & González-Sáiz, J.M. (2007). Optimisation of a headspace solid-phase microextraction with on-fiber derivatisation method for the direct determination of haloanisoles and halophenols in wine. *Journal of Chromatography A* 1143, 26–35

Pizarro, C.; Sáenz-González, C.; Pérez-del-Notario, N. & González-Sáiz, J.M. (2011). Development of a dispersive liquid–liquid microextraction method for the simultaneous determination of the main compounds causing cork taint and Brett character in wines using gas chromatography–tandem mass spectrometry. *Journal of Chromatography A* 1218, 1576–1584

Poláskova, P.; Herszage, J. & Ebeler, S. (2008) Wine flavour: chemistry in a glass. *Chemical Society Reviews* 37, 2478–2489

Portmann, M. (2000). Anatomie des organs des sens. In: La Dégustation. *Journal International des Sciences de la Vigne et du Vin,* nº Hors Série, pp. 10–13

Pozo-Bayón, M.Á. & Reineccius, G. (2009) Interactions between wine matrix macro-components and aroma compounds. In: *Wine Chemistry and Biochemistry*, MV. Moreno-Arribas and M.C. Polo (eds.). Springer, ISBN 978-0-387-74116-1

Prieto, A.; Basauri, O.; Rodil, R.; Usobiaga, A.; Fernández, L.A.; Etxebarria, N. & Zuloaga, O. (2010). Stir-bar sorptive extraction: A view on method optimisation, novel

applications, limitations and potential solutions. *Journal of Chromatography A* 1217, 2642–2666

Ramey, D.D.; Bertrand, A.; Ough, C.S.; Singleton, V.L. & Sanders, E. (1986). Effects of skin contact temperature on Chardonnay must and wine composition. *American Journal of Enology and Viticulture* 37 (2), 99-106

Rapp, A. & Mandery, H. (1986). Wine aroma. *Experientia* 42, 873–884

Rapp, A. & Versini, G. (1995). Influence of nitrogen compounds in grapes on aroma compounds of wines. In: *Food Flavors: Generation, Analysis and Process Influence*, George Charalambous (Ed.), 8th International Flavor Conference, Cos, Greece, 6-8 July 1994, Elsevier, pp. 1659–1694

Rapp, A.; Guntert, M. & Heimann, W. (1984). Characterization of the Riesling grape varieties. Investigation of volatiles of German white wine varieties: Riesling, Muller-Thurgan and Silvaner. *Vitis* 24, 139–150

Rasmussen, K.E. & Pedersen-Bjergaard, S. (2004). Developments in hollow fibre-based, liquid-phase microextraction. *Trends Analytical Chemistry* 23(1), 1–10

Razungles, A.; Baumes, R.L.; Dufour, C.; Sznaper, C.N., & Bayonove, C.L. (1998). Effect of sun exposure on carotenoids and C_{13}-norisoprenoid glycosides in Syrah berries (*Vitis vinifera L.*). *Sciences des Aliments* 18 (4), 361–373

Razungles, A.; Günata, Y.Z.; Pinatel, S.; Baumes, R.L. & Bayonove, C.L. (1993). Étude quantitative de composés terpéniques, norisoprenoides et de leurs précurseurs dans diverses variétés de raisins. *Sciences des Aliments* 13, 59–72

Regodon, J.A.; Pérez, F.; Valdés, M.E.; De Miguel, C. & Ramirez, M. (1997). A simple and efective procedure for selection of wine yeast strains. *Food Microbiology* 14, 247–254

Rezaee, M.; Assadi, Y.; Hosseini, M-R.M.; Aghaee, E.; Ahmadi, F. & Berijani, S. (2006). Determination of organic compounds in water using dispersive liquid–liquid microextraction. *Journal of Chromatography A* 1116, 1–9

Rezaee, M.; Yamini, Y. & Faraji, M. (2010). Evolution of dispersive liquid–liquid microextraction method. *Journal of Chromatography A* 1217, 2342–2357

Ribéreau-Gayon, P.; Glories, Y.; Maujean, A. & Dubourdieu, D. (2000). Varietal aroma. *In: Handbook of Enology. Volume 2 – The Chemistry of Wine and Stabilization and Treatments*, John Wiley & Sons Ltd., ISBN 0-471-97362-9, Chichester, England, pp. 187–206

Ridgway, K.; Lalljie, S.P. & Smith, R.M. (2007). Sample preparation techniques for the determination of trace residues and contaminants in foods. *Journal of Chromatography A* 1153, 36–53

Risticevic, S.; Niri, V.H.; Vuckovic, D. & Pawliszyn, J. (2009). Recent developments in solid-phase microextraction. *Analytical and Bioanalytical Chemistry* 393, 781–795

Romano, P.; Caruso, M.; Capece, A.; Lipani, G.; Paraggio, M. & Fiore, C. (2003). Metabolic diversity of Saccharomyces cerevisiae strains from spontaneous fermented grape musts. *World Journal of Microbiology and Biotechnology* 19, 311–315

Salinas, M.R.; Zalacain, A.; Pardo, F. & Alonso, G.L. (2004). Stir bar sorptive extraction applied to volatile constituents. Evolution during *Vitis vinifera r*ipening. *Journal of Agriculture and Food Chemistry* 52, 4821–4827

Sánchez-Palomo, E.; Díaz-Maroto, M.C. & Pérez-Coello, M.S. (2005). Rapid determination of volatile compounds in grapes by HS-SPME coupled with GC–MS. *Talanta* 66, 1152–1157

Schneider, R.; Charrier, F.; Moutounet, M. & Baumes, R. (2004). Rapid analysis of grape aroma glycoconjugates using fourier-transform infrared spectrometry and chemometric techniques. *Analytica Chimica Acta* 513, 91–96

Schneider, R.; Kotseridis, Y.; Ray, J.L.; Augier, C. & Baumes R. (2003) Quantitative determination of sulfur-containing wine odorants at sub parts per billion levels. 2. Development and application of a stable isotope dilution assay. *Journal of Agriculture and Food Chemistry 51,* 3243–3248

Sefton, M.A.; Francis, I.L. & Williams, P.J. (1993). The volatile composition of Chardonnay juices: A study by flavor precursor analysis. *American Journal of Enology and Viticulture* 44 (4), 359–369

Sefton, M.A.; Skouroumounis, G.K.; Massy-Westropp, R.A. & Williams, P.J. (1989). Norisoprenoids in *Vitis vinifera* white wine grapes and the identification of a precursor of damascenone in these fruits. *Australian Journal of Chemistry* 42, 2071–2084

Segurel, M.A.; Razungles, A.; Riou, C.; Trigueiro, M.G. & Baumes, R.L. (2005). Ability of possible DMS precursors to release DMS during wine aging and in the conditions of heat-alkaline treatment. *Journal of Agriculture and Food Chemistry* 53, 2637–2645

Setkova, L.; Risticevic, S. & Pawliszyn, J. (2007a). Rapid headspace solid-phase microextraction–gas chromatographic–time-of-flight mass spectrometric method for qualitative profiling of ice wine volatile fraction I. Method development and optimization. *Journal of Chromatography A* 1147, 213–223

Setkova, L.; Risticevic, S. & Pawliszyn, J. (2007b). Rapid headspace solid-phase microextraction-gas chromatographic–time-of-flight mass spectrometric method for qualitative profiling of ice wine volatile fraction. II: Classification of Canadian and Czech ice wines using statistical evaluation of the data. *Journal of Chromatography A* 114, 224–240

Simpson, R.F. (1978). 1,1,6-trimethyl-1,2-dihydronaphthalene: an important contributor to the bottle aged bouquet of wine. *Chemistry & Industry* 1, 37

Simpson, R.F. (1979). Some important aroma components of white wine. *Food Technology in Australia* 31, 516–522

Stone, H. & Sidel, J.L. (1998). Quantitative descriptive analysis: developments, applications, and the future. *Food Technology* 52, 48–52

Tankeviciute, A.; Kazlauskas, R. & Vickackaite, V. (2001). Headspace extraction of alcohols into a single drop. *Analyst* 126, 1674–1677

Tat, L.; Comuzzo, P.; Stolfo, I. & Battistutta, F. (2005). Optimization of wine headspace analysis by solid-phase microextraction capillary gas chromatography with mass spectrometric and flame ionization detection. *Food Chemistry* 93, 361–369

Tenenhaus, M.; Pages, J.; Ambrisine, L. & Guinot, C. (2005). PLS methodology to study relationships between hedonic judgements and product characteristics. *Food Quality and Preference* 16, 315–325

Tominaga, T. & Dubourdieu, D. (2000). Recherches sur l'arôme variétal des vins de *Vitis vinifera* L. cv. Sauvignon blanc et sa genèse à partir de précurseurs du raisin. *Revue des OEnologues* 97, 22–28

Tominaga, T.; Darriet, P. & Dubourdieu, D. (1996). Identification de l'acétate de 3-mercaptohexanol, composé à forte odeur de buis, intervenant dans l'arôme des vins de Sauvignon. *Vitis* 35 (4), 207–210

Tominaga, T.; Furrer, A.; Henry, R. & Dubourdieu, D. (1998a). Development of a method for analyzing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. cv. Sauvignon blanc. *Journal of Agriculture and Food Chemistry* 46 (3), 1044–1048

Tominaga, T.; Furrer, A.; Henry, R. & Dubourdieu, D. (1998b). Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour and Fragrance Journal* 13, 159–162

Tredoux, A.; de Villiers, A.; Májek, P.; Lynen, F.; Crouch, A. & Sandra P. (2008). Stir bar sorptive extraction combined with GC-MS analysis and chemometric methods for the classification of South African wines According to the volatile composition. *Journal of Agricultural and Food Chemistry 56,* 4286–4296

Usseglio-Tomasset, L. (1983). Influence de la temperature de conservation sur les caractéristiques physico-chimiques et organoleptiques des vins (vins aromatiques). *Bulletin de l'OIV* 623, 19–34

van Leeuwen, C.; Friant, Ph.; Choné, X.; Trégoat, O.; Koundouras, S. & Dubourdieu, D. (2004). The influence of climate, soil and cultivar on terroir. *American Journal of Enology and Viticulture* 55, 207–217

Versini, G. (1985). On the aroma of "Traminer aromatico" or "Gewürztraminer" wine. *Vignevini* 12, 57–65

Versini, G.; Orriols, I. & Dalla Serra, A. (1994). Aroma components of Galician Albariño, Loureira and Godello wines. *Vitis* 33, 165–170

Vilanova, M. & Martínez, M.C. (2007). A first study of aromatic compounds of red wine from *Vitis vinifera* cv. Castañal grown in Galicia (NW Spain). *European Food Research & Technology* 224, 431–436

Vilanova, M. & Masneuf-Pomarède, I. (2005a). Characterization of yeast strain from Rias Baixas (NW Spain) and the contribution to the fermentation of Albariño wine. *Annals of Microbiol*ogy 55, 23–26

Vilanova, M. & Masneuf-Pomarède, I. (2005b). Comparison of volatiles in Albariño wine produced by *Saccharomyces cerevisiae* strain. *Italian Journal of Food Science* 17 (2), 221–227

Vilanova, M. & Sieiro, C. (2006a). Contribution by *Saccharomyces cerevisiae* yeast to fermentative flavour compounds in wines from cv. Albariño. *Journal of Industrial Microbiology and Biotechnology* 33 (11), 929–933

Vilanova, M. & Sieiro, C. (2006b). Determination of free and bound compounds in Albariño wine. *Journal of Food Composition and Analysis* 19(6-7), 694–697

Vilanova, M., Genisheva, Z.; Bescansa, L., Masa, A. & Oliveira, J.M. (2009). Volatile composition of wines from *Vitis vinifera* Agudelo, Serradelo and Blanco lexítimo from Betanzos (NW Spain). *Journal of the Institute of Brewing* 115 (1), 35–40

Vilanova, M., Genisheva, Z.; Masa, A.; Oliveira, J.M. (2010). Correlation between volatile composition and sensory properties in Spanish Albariño wine. *Microchemical Journal* 95, 240–246

Vilanova, M.; Cortés, S.; Santiago, J.L., Martínez, C. & Fernández, E. (2007a). Aromatic compounds in wines produced during fermentation: effect of three red cultivars. *International Journal of Food Properties* 10, 867–975

Vilanova, M.; Cortés, S.; Santiago, J.L.; Martínez, C. & Fernández, E. (2008). Potent contribution of some grape-derived aromatic compounds to the primary aroma in red wines from cv. Caíño Tinto, cv. Caíño Bravo and cv. Caíño Longo grapes. *Journal of Agricultural Sciences* 146 (3), 325–332

Vilanova, M.; Zamuz, S.; Vilariño, F. & Sieiro, C. (2007b). Effect of *terroir* on the volatiles of *Vitis vinifera* cv. Albariño. *Journal of the Science of Food and Agriculture* 87, 1252-1256

Voirin, S.; Baumes, R.L.; Bitteur, S.M.; Günata, Y.Z. & Bayonove, C.L. (1990). Novel monoterpene disaccharide glycosides of *Vitis vinifera* grapes. *Journal of Agriculture and Food Chemistry* 38 (6), 1373–1378

Watanabe, N.; Messerer, M. & Winterhalter, P. (1997). Uncommon glycoconjugates of 2-phenylethanol from rose flowers and Riesling wine. *Natural Products Lett*ers 10, 39–42

Weldegergis, B.T. & Crouch, A.M. (2008). Analysis of volatiles in Pinotage wines by stir bar sorptive extraction and chemometric profiling. *Journal of Agriculture and Food Chemistry 56*, 10225–10236

Weldegergis, B.T.; Crouch, A.M.; Gorecki, T. & de Villiers, A. (2011) Solid phase extraction in combination with comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry for the detailed investigation of volatiles in South African red wines. *Analytica Chimica Acta* 701, 98–111

Williams, P.J.; Strauss, C.R. & Wilson B. (1980). Hydroxylated linalool derivatives as precursores of volatile monoterpenes of muscat grapes. *Journal of Agriculture and Food Chemistry* 28, 766–771

Wilson, B.; Strauss, C.R. & Williams, P.J. (1986). The distribution of free and glycosidically-bound monoterpenes among skin, juice, and pulp fractions of some white grape varieties. *American Journal of Enology and Viticulture* 37 (2), 107–111

Xiao, Q.; Yu, C.; Xing, J. & Hu, B. (2006). Comparison of headspace and direct single-drop microextraction and headspace solid-phase microextraction for the measurement of volatile sulfur compounds in beer and beverage by gas chromatography with flame photometric detection. *Journal of Chromatography A* 1125, 133–137

Xu, L.; Basheer, C. & Lee, H.K. (2007). Developments in single-drop microextraction. *Journal of Chromatography A* 1152, 184–192

Zalacain, A.; Alonso, G.L.; Lorenzo, C.; Iñiguez, M. & Salinas, M.R. (2004). Stir bar sorptive extraction for the analysis of wine cork taint. *Journal of Chromatography A* 1033, 173–178

Zalacain, A.; Marín, J., Alonso, G.L. & Salinas, M.R, (2007). Analysis of wine primary aroma compounds by stir bar sorptive extraction. *Talanta* 71, 1610–1615

Zamuz, S. & Vilanova, M. (2006a). Volatile composition of the *Vitis vinifera* Albariño musts according to geographic area from Rías Baixas AOC (Spain). *Italian Journal of Food Science* 3 (18), 323–328

Zamuz, S. & Vilanova, M. (2006b). Volatile compounds after spontaneous fermentation of must from Vitis vinifera cv Albariño grapes cultived in different origins from Rías Baixas AOC Spain. *Flavour and Fragrance Journal* 21, 743–748

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