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Review

Analysis of organic sulfur compounds in wine aroma

M. Mestres, O. Busto, J. Guasch*

Departament de Química Analítica i Química Orgànica (Unitat d'Enologia, CeRTA), Facultat d'Enologia de Tarragona, Universitat Rovira i Virgili, Avda. Ramón y Cajal 70, E-43005 Tarragona, Spain

Abstract

Sulfur-containing compounds in wines have been extensively studied because of their effect on wine aroma. The aim of this paper was to give an overview on the analytical methods developed to determine them in wines with special emphasis on gas chromatographic methods, as well as the results obtained. In addition, the problems occurring in application of the common extraction procedures, such as liquid–liquid extraction, static and dynamic headspace and solid-phase microextraction, are presented and discussed.

Keywords: Reviews; Wine; Food analysis; Sample handling; Sulfur compounds; Aroma compounds; Organosulfur compounds

Contents

*Corresponding author.
E-mail address: qaenol@fe.urv.es (J. Guasch)

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1. Introduction

Flavour is one of the most important determinants of food and beverage quality, since the interaction of aromatic substances with the senses of smell and taste leads to consumer acceptance or rejection. In some cases, the presence of a single compound is sufficient to give the characteristic aroma of a product (key or character impact substance) but, in general, the aroma of foods is influenced by several different compounds. Among these, sulfur-containing structures are an important class both due to their abundance and aromatic impact. In fact, about 10% of the volatile components detected in foods and beverages are sulfur compounds [1] and their character impact is so noticeable as to give characteristic notes to different products [1,2].

When degraded, these structures, such as cystine, cysteine, methionine, glutathione and some vitamins, become a source of different kinds of sulfur compounds. Their degradation may occur by enzymatic [3–5] or non-enzymatic routes in which the rate can be greatly influenced by temperature and light [6–11]. However, this natural source of sulfur may be influenced by addition of additives to foods and beverages. In fact, their degradation may originate further sulfurated compounds.

From organoleptic perspectives, these compounds have different olfactory qualities depending on the position of the sulfur atom in the molecule [1]. In addition, their concentration has a great influence on sensory properties, often being strongly dependent on threshold values, normally low. Therefore low concentrations may give high odour intensities. Some of these aromas have been identified as favourable character-impact substances in different foods such as meat [1,7], coffee [1,12], corn [13], oranges [14], yellow passion fruit [1,15], truffles [1,16], allium species [1,5] and beer [17–20]. On the contrary, these compounds are sometimes responsible for offensive odours (off-flavours), and their presence indicates incorrect conditions of preparation and storage [21]. From an oenological point of view, both aspects have to be taken into account since off-flavours caused by sulfur-containing compounds cause some of the major defects in the quality of wine aroma [22–26] while other favourable ones may exalt the typical notes of some varietal wines [27–32].

2. Sulfur compounds in wines

Wine is a hydroalcoholic solution containing hundreds of compounds that come from grapes or result during winemaking and storage. Several of these compounds affect wine aroma [33–39] which, besides being a parameter of quality, act as a «fingerprint» for each wine variety. In fact, some of these odour compounds are characteristic of certain varieties whereas the concentration of other compounds, although present in all wines, varies according to the type of wine. Chemically related to sulfur structures, these compounds therefore have a great sensorial impact and play a more important role in the flavour of wines.

The sulfur compounds found in wines are classified in five different families according to their structure: thiols, sulphides, polysulphides, thioesters and heterocyclic compounds. However, some authors [40,41] arbitrarily divide these into two groups, according to their volatility: those with a boiling point below 90°C (volatile compounds) and above 90°C (less volatile compounds). The latter classification is of more practical use and applicable when the analytical technique involves the consideration of the boiling points of analytes rather than their chemical composition.

Table 1 provides data dealing with the average contents and odours of the sulfur compounds found in wines. It is clear from inspection, that different concentrations of some sulfur compounds have been found for some wine varieties and for wines, which have either disagreeable odours or great cloudiness.

2.1. Influence of sulfur compounds on wine aroma

A variety of sensory impressions are possible for wines depending on their unique concentrations of sulfur compounds, their aromatic properties and synergist–antagonist effects. In general, the aromatic contributions of these compounds are considered detrimental to wine quality. As seen in Table 1, the odour of these compounds can be described with terms such as cabbage, garlic, onion or rubber, which allude to their negative effects on wine aroma. However, there are some sulfur compounds, with more specific descriptors, which are typical of some varieties and which contribute actively to the varietal aroma of these wines. Examples include of this are

Table 1
Content and odours of sulfur compounds in wines[a]

	Sulfur compound	Contents	Odour	Reference
1	Hydrogen sulphide	nd-370 μg/L	rotten egg, decaying seaweed, reduced taste	[24,26,33,34,38,40,54,74,75,76,78, 81,82,87,94,96,97]
2	Methanethiol	nd-16 μg/L*	putrefaction, cooked cabbage, reduced taste	[24,26,40,54,74–77,78,81–83,87,97]
3	Carbonyl sulphide	nd-2.4 μg/L	odourless	[24,74,77,78,95]
4	Ethanethiol	nd-5 μg/L* 0–50 μg/L (Me)	onion, rubber, fecal, putrefaction (leek, garlic, onion)	[22,24,26,33,70,74–76,78,81–83,87,96,97]
5	Dimethyl sulphide	nd-50 μg/L 0–474 μg/L (Ri)	cabbage, asparagus, corn, molasses low concentrations: herbaceous	[22,24,26,33,38,40,43,44–46,54,70,74–76, 78–81,82,85–87,95,96,98–102]
6	Carbon disulphide	nd-18 μg/L*	rubber, chokingly repulsive, cabbage	[24,40,70,74–76,78,80–82,86,87,95–97,100]
7	Ethylmethyl sulphide	traces	–	[33]
8	Diethyl sulphide	nd-10 μg/L*	garlic	[22,33,40,70,74–76,78,86,87,101,103]
9	Dipropyl sulphide	traces	–	[33]
10	Methyl-n-propyl sulphide	nd-2.7 μg/L	sulfurous	[75,76,85– 87]
11	Ethylpropylsulphide	traces	–	[33]
12	Methyl thioacetate	nd-20 μg/L nd-115 μg/L (PN) 5.1–85 μg/L**	sulfurous	[70,75,76,80–82,83,85–87,91,96,97,100]
13	Ethyl thioacetate	nd-7 μg/L nd-56 μg/L (PN) 3.2–180 μg/L **	sulfurous	[75,76,80,82,83,85–87,91,96]
14	Dimethyl disulphide	0–22 μg/L* 0.8–8.2 μg/L ** 3–6.5 μg/L***	cabbage, cooked cabbage, sulfurous, sickly, onion	[22,24,33,40,54,74–76,79,80,82,83, 85–87,96,97,100,101,103]
15	Ethylmethyl disulphide	nd-0.01 μg/L nd-1.4 μg/L**	–	[33,74,81–83]
16	Diethyl disulphide	nd-3 μg/L 0–85 μg/L (Cs) 0–82 μg/L (Me)	bad smelling, onion	[26,33,75,76,81–83,85–87,97,101,103]
17	2-mercaptoethanol	nd-400 μg/L (higher contents in no VV) 113–179 μg/L***	boxer, poultry, farmyard, alliaceous	[41,52,70,93,97,104,105]
18	Methylthioethanol	88–139 μg/L (Chd) 7–14 μg/L (Cs) 25–98 μg/L(Chn) 5–13 μg/L (Se) 61–66 μg/L***	french bean	[41,52]
19	3-methylthio-1-propanol (methionol)	145– 2000 μg/L 2330–3500 μg/L (Chd) 140–330 μg/L (Cs) 1640–2210 μg/L (Chn) 1340–1720 μg/L (Se) 224–5655 μg/L ** 500–3266 μg/L***	potato, soup or meat like, cauliflower, cooked cabage	[24,33,40,41,43,52,63,64,70,91,96,97, 101,103,106]
20	4-methylthio-1-butanol	nd-181 μg/L* 35–66 μg/L***	onion, garlic, earthy, alliaceous	[40,41,52,106]
21	Methyl-3-methylthiopropionate	–	sulfurous	[33,70,96]
22	Ethyl-3-methylthiopropionate	0–10 μg/L 0–14 μg/L* 0.9–14.3 μg/L** 4–7 μg/L***	sulfurous, metallic	[40,41,52,64,70,91,101,103,106]
23	3-methylthio-1-propanal (methional)	0–42ppb μg/L* 0–57.5 μg/L***	onion, meat, mashed potatoes, soup	[24,40,70]

Table 1. Continued

	Sulphur compound	Contents	Odour	Reference
24	3-methylthiopropyl acetate	traces-1 μg/L 2–14 μg/L* 0–17 μg/L***	mushroom, garlic	[33,40,41,52,64,70,91,96,101,103,106]
25	Dihydro-2-methyl(2H)tiophen-3-one	0,1–1 mg/L (V) 3.2–190 μg/L***	sulfurous	[52,96,106]
26	2-methyltetrahydrothiophan-3-one	18.7–61.7 μg/L 27–268 μg/L* 14.8–237.2 μg/L** 131–478 μg/L*** 10.4–28.4 μg/L (Chd) 41–11.2 μg/L (S) 3.3–11.9 μg/L (Chn) 6.8–61 μg/L (Se)	metallic, natural gas	[33,40,41,91,97,103]
27	3-ethylthio-1-propanol	11–88 μg/L	–	[64,96]
28	Benzothiazol	0–6 μg/L 0–13 μg/L* 0.7–13.8 μg/L** 0–30 μg/L***	rubber	[33,40,41,52,70,91,96,101,103,106]
29	4-mercapto-2,5-dimethyl(2H)-thiophen-3-one	typical of VL	strawberry, sulfurous	[29]
30	4-mercapto-4-methylpentan-2-one	0–34 ng/L (SBl) 4–10 μg/L (SBo) 4–24 μg/L (SS) <10 μg/L (Ge)	box tree, guava aroma, cat urine, broom, passion fruit	[28,31,42,56,57,107–109]
31	3-mercaptohexanol	10–5000 ng/L 7400–12800 μg/L (SBo) 733–3400 μg/L (SS)	fruity, animal, grape, box tree, broom, passion fruit	[30–32,57,72]
32	3-mercapto-2-methylpropan-1-ol	250–10000 ng/L	fruity, animal, sweat, broth	[32,72]
33	4-mercapto-4-methylpentan-2-ol	18–22 μg/L (SBo) 1–20 μg/L (SS)	citric, passion fruit, box tree, broom	[30,31,57]
34	3-mercapto-3-methylbutan-1-ol	78–97 μg/L (SBo) 34–134 μg/L (SS)	coocked leeks	[30,31]
35	cis-2-methiolan-3-ol	0.9–29.4 μg/L* 3.9–94.8 μg/L**	odourless	[40,66,67,91,97]
36	trans-2-methiolan-3-ol	0.4–22.8 μg/L 3.8–72.1 μg/L** 3.9–9.5 μg/L (Chd) 23.6–47 μg/L (Chn) 17.4–37.8 μg/L (Se)	onion, young onion	[34,40,41,66,67,91,97]
37	2-methylthiophene	0–5 μg/L*	sulfurous	[40,70]
38	bis(2-hydroxyethyl) disulphide	21–1400 μg/L (higher contents in no VV)	odourless	[93]
39	3-methylthiopropionic acid	0–70 μg/L (WW) 1–140 μg/L (RW) 85–310 μg/L ***	butter, rancid	[52,69,105]
40	Dimethylsulfoxide	363–1448 μg/L ***	odourless	[52]
41	2-mercaptoethyl acetate	23–134 μg/L	roasted meat	[62]
42	3-mercaptoppropyl acetate	3–32 μg/L	roasted meat	[62]
43	3-mercaptohexyl acetate	1–200 ng/L 275–724 μg/L (SBo) 212–777 μg/L (SS)	box tree, passion fruit, broom	[31,32,71]
44	5-(2-hydroxyethyl)-4-methylthiazol	5–50 μg/L	medicinal, cacao	[67]
45	N-3-methylthiopropyl acetamide	0–2430 μg/L	odourless	[69,96,105]
46	2-((methylthio)-methylthio)-ethanol	10–50 μg/L	cauliflower, garlic (at low concentration)	[67,97]

[a] *: higher content in reduced wine; **: wine with disagreeable odour; ***: higher content in cloudy wine Me: Merlot; Ri: Riesling; PN: Pinot Noir; Cs: Cabernet sauvignon; Chd: Chardonnay; Se: Semillon; Chn: Chenin; V: Verdejo; S: Sauvignon; SBl: Sauvignon Blanc; SBo: Sauvignon Bordeaux; SS: Sauvignon Sancerre; Ge: Gewürztraminer; VL: Vitis labrusca; VV: Vitis vinifera; WW: white wine; RW: red wine; nd: not detected

the odour of strawberry for 4-mercapto-2,5-dimethyl(2H)thiophen-3-one [29], box tree for 3-mercaptohexylacetate [27], cat urine for 4-mercapto-4-methylpentan-2-one [42] or cooked leeks for 3-mercapto-3-methylbutan-1-ol [31]. Furthermore, low concentrations of some sulfur compounds, such as dimethyl sulphide or carbon disulphide, reportedly produce satisfactory wine aromas [43–46].

Table 2 shows typical perception thresholds for some of the most important sulfur compounds in wines. It can be seen that these values are, in general, at μg/l levels although the thresholds may be at ng/l levels, as is the case of SH_2 and 4-mercapto-4-methylpentan-3-one, depending on the original matrix.

2.2. Origin of sulfur compounds in wines

Several investigations on the formation of sulfur compounds in wines have been carried out, pro-

Table 2
Limits of perception of some sulfur compounds in different matrices[a]

Sulfur compound limits of perception	References			
	wine	water	others	
Hydrogen sulphide	1 ng/L-150 μg/L	5–10 μg/L	0.8 μg/L (HS)	[24,34,38,74,78,94,97,110]
Methanethiol	–	0.02–2 μg/L	0.3 μg/L (HS) 0.2–81 ng/L (A)	[24,26,78,110]
Ethanethiol	1.1 μg/L	8 ng/L	1–10 μg/L (B) 0.1 μg/L (HS)	[22,24,26,77,78]
Dimethyl sulphide	10–160 μg/L	0.3–10 μg/L	5–10 μg/L (HS)-50–60 μg/L (B)	[22,24,26,40,45,78,101,111]
Carbon disulphide	–	–	50–500 ng/L (A)	[24,95]
Diethyl sulphide	0.93–18 μg/L	–	6 μg/L (HS) 1–30 μg/L (B)	[22,40,78,101,103]
Dimethyl disulphide	20–45 μg/L	0.06–30 μg/L	2.5 μg/L (HS) 3–50 μg/L (B)	[22,24,40,78]
Diethyl disulphide	4.3–40 μg/L	–	20 μg/L (HS) 0.4 μg/L (B)	[22,26,40,101,103]
2-mercaptoethanol	0.13–10 mg/L	–	0.1–10 mg/L	[41,52,67,93,104]
Methylthioethanol	–	–	250 μg/L (HS)	[52]
3-methylthio-1-propanol (methionol)	1.2–4.5 mg/L	–	1.2 μg/L (HS) 500 μg/L (B)	[24,41,101,103,112]
4-methylthio-1-butanol	0.1 mg/L	–	0,08–1 mg/L (HS)	[41,52,67]
Ethyl-3-methylthio propionate	0.3–1 mg/L	–	300 μg/L (HS)	[41,91,101,103]
3-methylthio propan-1-al	–	0.2–50 μg/L	250 μg/L (B)	[24]
3-methylthiopropyl acetate	50–115 μg/L		50 μg/L (HS)	[41,52,101,103]
Dihydro-2-methyl(2H) tiophen-3-one	–	–	70 μg/L (HS)	[52]
Benzothiazole	50–350 μg/L		50 μg/L (HS)	[52,101,103]
4-mercapto-4-methyl pentan-2-one	0.8–3 ng/L	0.1 ng/L	0.6 ng/L (HS)	[28,31,42,107,108,111]
3-mercaptohexanol	–	12–15 ng/L	60 ng/L (HS)	[31,32]
3-mercapto-2-methyl-propan-1-ol	–	3000 ng/L	–	[32]
4-mercapto-4-methyl-pentan-2-ol	–	–	55 ng/L (HS)	[31]
3-mercapto-3-methyl butan-1-ol	–	–	1.5 μg/L (HS)	[31]
trans-2-methiolan-3-ol (tiofanol)	100–500 μg/L	–	–	[41]
3-methylthiopropionic acid	–	–	50 μg/L (HS)	[52]
2-mercaptoethyl acetate	–	–	65 μg/L (HS)	[62]
2-mercaptopropil acetate	–	–	35 μg/L (HS)	[62]
3-mercaptohexyl acetate	–	2,3 ng/L	4 ng/L (HS)	[31,32,71]
5-(2-hydroxyethyl)-4-metilthiazol	0.1–1 mg/L			[67]
2((methylthio)-methylthio)-ethanol			0.1–1 mg/L	[67]

[a] HS: Hydroalcoholic solution; B: Beer; A: Air.

viding an explanation or suggestions for their origin. In general, their presence in wines originates from two main processes that are either enzymatic or non-enzymatic [23,24,47–52]. The first one involves the degradation of sulfur-containing amino acids, the formation of fermentation products and the metabolism of some sulfur-containing pesticides. Non-enzymatic pathways include photochemical, thermal and other chemical reactions of sulfur compounds during winemaking and storage.

The most widely studied origins of different sulfur compounds involves reduction reactions catalyzed by light, to produce unpleasant flavours called «light tastes» or «reduced tastes» in oenological slang [53,54]; the degradation of sulfur-containing pesticides [23–25]; and, the yeast metabolism of some amino acids. For example, it is well known that methionine is metabolized with formation of its fusel alcohol (3-methylthio-1-propanol or methionol), its acetate (3-methylthiopropyl acetate), its ethyl ester [ethyl (3-methylthio) propionate] and 3-ethylthio-1-propanol [33,34,38,55]. The origins of 4-methylthio-1-butanol and 2-mercapto-1-ethanol are believed to be similar to that of methionol but starting from homomethionine and cysteine, respectively [36,38]. Recently, a compound found to exert a key role in Sauvignon wine aroma has been identified as 4-methyl-4-mercaptopentan-2-one, and it seems to have a *S*-cysteine conjugate as precursor [56,57]. It has also been demonstrated that yeasts can utilise sulfur-containing pesticides to form sulfur compounds such as CS_2, H_2S or thiols. These thiols are easily oxidizable to disulphide forms and they can also react with other wine aroma compounds, giving rise to other off-flavours [23–25].

3. Analysis of sulfur compounds in wines

Three of the main problems encountered in analysis of sulfur compounds in wine are the complexity of the sample matrix, the low concentration levels that must be determined, and the highly reactive nature of these compounds.

The literature reports the application of several analytical techniques. Thiols may be determined with the sulfur specific ion electrode [58–60], but more practical spectrophotometric methods are also available. In fact, they can be detected colorimetrically using methylene blue [58–60] or 5,5′-dithiobisnitrobenzoic acid [53,61] as reagents. Dimethylsulphide has been measured after reaction with sodium nitroprusside [44]. Some of these procedures use a specific trap, such as cellulose, zinc acetate and cadmium hydroxide [58–60], to collect the sulfur analytes before the spectrophotometric assay. At present, *p*-hydroxymercuribenzoate (pHMB) is the most widely used reagent trap. It specifically reacts with thiol groups, which can be released with addition of an excess of glutathione or cysteine [30–32,62].

Nowadays gas chromatography (GC) is the most widely used technique due to its sensitivity, specificity and reliability. However, separation and preconcentration steps are usually required before the chromatographic analysis, due to the low concentrations of sulfur compounds in wines.

3.1. Sampling and concentration techniques

Table 3 provides examples of the sampling and concentration techniques used to analyse sulfur compounds in wines including techniques of liquid–liquid extraction, static headspace, dynamic headspace and, more recently, solid-phase microextraction.

3.1.1. Liquid–liquid extraction

Liquid-liquid extraction (LLE) with organic solvents has been widely used in the analysis of wine aroma. Using azeotropic mixtures with more than one solvent, it is possible to modify the polarity range of the compounds extracted and improve the efficiency. Generally, the extraction efficiency increases when a salt is added, but this technique can only be used for analytes with boiling points that are not too low so as to prevent losses during sample handling. The use of large solvent volumes, which can be toxic and/or environmental pollutants, and time consuming are the main disadvantages of this technique [63–67]. Nevertheless, this approach was applied during the first studies and is still in use [40,41,68–70].

As can be seen in Table 3, ethyl acetate and a mixture of pentane–dichloromethane, are the preferred solvent used. The extracts are then concen-

Table 3
Sampling and concentration techniques for determining sulfur compounds in wines[a]

	Parameters				Ref.	Compounds
	solvent	C.F.	Time	others		
LLE	DCM	40000	–	5% w/v Na_2SO_4	[63]	19
	C5:DCM 2:1	(V)	–	–	[64]	19, 22, 24, 27
	C5:DCM 2:1	–	–	–	[65]	19
	F11:DCM (9:1)	12.5 (V)	20 h (C.E.))	–	[66]	35, 36
	F11:DCM (9:1)	–	15 h (C.E.)	–	[67]	17, 20, 35, 36, 44, 46
	F11	333 (V)	24 h (C.E.)		[106]	19, 20, 22, 24, 25, 28
	DEE:C5 1:1	100 (N_2)	5 min (×3)	antioxidant	[68]	5, 8, 14, 15, 19, 22, 23, 24, 26, 28
	EtAc	100 (N_2)	5 min (×2)	10% w/v Na_2SO_4	[93,105]	17, 19, 38, 39, 45
	EtAc	100 (N_2)	5 min (×2)	10% w/v Na_2SO_4	[41]	17, 18, 19, 20, 22, 24, 26, 28, 36
	DEE:C5 (1:9)	8–9	5 min (×3)	pHMB and P&T	[42]	30
	EtAc	100 (N_2)	5 min (×2)	10% w/v Na_2SO_4	[69]	39, 45
	C5: DCM 2:1	1000 (K-D)	–	–	[70]	4, 5, 6, 8, 12, 13, 14, 17, 19, 20, 21, 22, 23,24, 26, 28, 37
	DCM:C5 1:2)	5000 (N_2)	5 min (×3)	pHMB	[28]	30
	DCM	150000	5 min (×2)	pHMB	[71]	43
	EtAc	100 (N_2)	5 min (×2)	10% w/v Na_2SO4	[29]	29
	DCM	1000	5 min (x2)	pHMB/anionic column	[31]	30, 31, 33, 34, 43
	DCM	50000 (N_2)	3 times	pHMB (distillation)	[32,72]	31, 32, 43
	DCM	100000	5 min×2	pHMB	[30]	31, 33, 34
	DCM	1500 (N_2)	3 times	pHMB	[62]	41, 42
	liquid/HS	time	T[a]	others		
SHS	25 mL/25 mL	25 min	40°C	40% w/v $(NH_4)_2SO_4$	[43]	5
	275 mL/75 mL	24 h	20°C	–	[40]	1, 2, 5, 6, 8, 14
	300 mL/75 mL	24 h	20–25°C	–	[78]	1, 2, 3, 4, 5, 6, 8, 14
	200 mL/50 mL	72 h	20°C	–	[77]	1, 2, 4, 5, 6
	50 mL/72 mL	30 min	–	–	[26]	1, 3, 4, 5, 6, 14
	15 mL/5 mL	2 h	63°C	–	[74]	1, 2, 3, 4, 5, 6, 8, 14, 15
	10 mL/10 mL	2 h	60°C	–	[75,76]	1, 2, 4, 5, 6, 8, 10, 12, 13, 14, 15, 16
	300 mL/75 mL	24 h	20°C		[52]	17, 18, 19, 20, 22, 24, 26, 28, 39, 40
	sorbent	T[a], time	sample	purge flow		
DHS	Ch 101	20°C, 15 min	200 mL	20 mL/min (N_2)	[79]	5, 14
	Ch 101	20°C, 10 min	200 mL	50 mL/min (N_2)	[80]	1, 2, 5, 6, 12, 13, 14
	Te/Ch102	25°C, 25 min	–	–	[23,111]	2, 4, 5, 6, 8, 12, 13, 14
	liqN	32°C	100 ml	30 min (equilibration))	[100]	5, 6, 12, 14, 37
	Cryo	–	–	–	[108]	30
	Te	–	3 mL	10 mL/min	[83]	2, 4, 12, 13, 14, 15, 16
	CPSil8-CB/liqN	70°C, 30 min	8 mL (extract)	15 mL/min (H_2)	[42]	30
	pHMB (92°C)	92°C, 120 min	500 mL	785 mL/min (N2)	[28]	31, 32, 43
	Cryo/PoropackQ	60°C		45 min (presampling)	[81,82]	1, 2, 4, 5, 6, 12, 13, 14, 15, 16
	fiber	T[a], time	liquid/HS	others		
SPME	CW/DVB	–, 30 min	5 mL/5 mL	SHS sampling, NaCl	[84]	–methyl isothiocyanate
	PAc, PDMS	30°C, 15 min	25 mL/25 mL	SHS sampling, NaCl	[85,75]	5, 6, 8, 10, 12, 13, 14, 16
	CAR/PDMS	25°C, 30 min	25 mL/25 mL	SHS sampling, NaCl	[86]	5, 6, 8, 10, 12, 13, 14, 16
	CAR/PDMS	25°C, 30 min	50 mL/25 mL	SHS sampling, NaCl	[87]	1, 2, 4, 5, 6, 8, 10, 12, 13, 14, 16

[a] Ref: Bibliographic references; Compounds: compounds analysed with each technique. LLE: liquid-liquid extraction. DCM: dichloromethane; C5: pentane; DEE: diethyl ether; pHMB: p-hydroxymercuribenzoate; EtAc: ethyl acetate; F11: freon 11; C.F.: Concentration factor; Concentration Methods: V(Vigreux), K-D (Kuderna-Danish), N_2 (N_2 stream). SHS: static headspace. liquid/HS: liquid and headspace volumes; time: equilibration time; T[a]: equilibration temperature. DHS: dynamic headspace. T[a], time: purge temperature and purge time; Sample: sample volume; Ch 101: Chromosorb 101; Ch 102: Chromosorb 102; Te: Tenax; Cryo: cryogenic trap; liqN: liquid nitrogen. SPME: solid-phase microextraction. Fiber: Stationary phase CW/DVB: Carbowax/Divinylbenzene; PAc: polyacrylate; PDMS: Polydimethylsiloxane; Carboxen: Carboxen/PDMS

trated by using N_2 stream or fractionation columns. Recently, pHMB solutions have been used to specifically extract thiols from wine. In some studies, these solutions are used to isolate the volatile thiols from a dichloromethane extract of wine [30,31,42,71] while in others, these compounds are directly extracted from wine by combining low-temperature vacuum distillation with a specific chemical trap of pHMB [32,72]. In both cases, the thiols are then released by adding an excess of glutathione or cysteine.

3.1.2. Static headspace

Headspace is the gas phase over a liquid or solid sample, which is placed in a septum-closed heated vial. After an equilibration time, the volatile compounds disperse in the gas phase at a concentration, which reflects their vapour pressure. A good way of analysing aroma is to determine the compounds in this gas phase since they are the most volatile and they can interact with our sense of smell.

This is a simple solvent-free technique in which sample handling is minimal. However, due to the absence of a concentration step, its sensitivity is low. In addition, only compounds whose boiling points are not very high can be analysed. In some cases, these problems can be solved by moderate heating of the liquid or solid sample, and/or increasing its ionic strength [43,73–76].

The static headspace procedure has been successfully applied to the analysis of various volatile sulfur compounds in wines [26,40,74–78].

3.1.3. Dynamic headspace

As with the previous technique, this procedure employs a solid or liquid sample that contacts a gas phase in a chamber. In this case, a chamber is purged using a gas flow carrying volatile headspace components to a trap, where they are adsorbed and concentrated. After this process, a desorption step is required, which is, in general, a thermal desorption step. Commercial purge-and-trap (PT) equipment is an example of this sampling procedure and, usually, the desorption and cryofocusing steps are automatic and the sample is injected directly into the GC system.

To improve the efficiency of the technique for analysing some liquid samples, carrier gas can be bubbled through a sample to help release more compounds into the analytical headspace. The sample can also be heated. Good sensitivity and selectivity are achieved when a suitable sampling time and an appropriate trap are applied. One restriction may derive from the limited adsorption capacity (breakthrough) of the trap, and the need for it to be replaced when some compounds are irreversibly adsorbed, giving rise to memory effects.

The trap used may be a solid sorbent, a liquid solution, or a cold trap. For the analysis of volatile sulfur compounds the adsorbents such as Chromosorb 101 [79,80], Chromosorb 102 [23], PoropackQ (80–100 mesh) [81,82], Tenax [16,23,70] and CPSil 8-CB (Chrompack) [42] are currently used. In some cases, the trap is kept at low temperatures to increase the adsorption efficiency (Table 3). Solutions of pHMB have been used specifically as a liquid trap for thiols, which are released by adding gluthatione or cysteine [28].

In general, this technique may be considered an alternative to the static headspace, good results have been obtained in its application [16,23,42,70,81,82].

3.1.4. Solid-phase microextraction

Solid-phase microextraction (SPME) is a recently developed *solventless* technique that uses a polymer-coated fibre to extract and concentrate analytes from the matrix. The sample is then directly transferred to the injector port of a GC system equipped for thermal desorption and analysis. The SPME unit consists of a length of fused-silica fibre coated with different phases and bonded to a stainless steel plunger of a modified syringe. The technique is very simple since it only involves immersing the fibre into either the liquid sample or the gas headspace above the sample to extract and concentrate the analytes on the fibre. In comparison with other extraction techniques, this is a ‘‘solvent-free’’ technique, which requires minimum sample handling. In addition, it shortens the concentration and extraction time while facilitating the analysis of either gas, liquid or solid samples.

In the analysis of sulfur compounds in wines, fibres coated with different sorbents have been assayed including Carbowax–divinylbenzene [84], polydimethylsiloxane [75,85], polyacrylate [85] and Carboxen–polydimethylsiloxane [86–88]. Variables

such as ionic strength, temperature and time of extraction, stirring and volume of the sample must be optimised. The results obtained using this approach are good although the repeatability is low for some kinds of fibre. Furthermore, due to the extraction efficiency of this technique, the matrix interferences of wine must be considered and, if the fibre is immersed in the liquid during sampling, these interferences are greater [88].

3.2. Chromatographic analysis

Due to its sensitivity, good separation capability and reliability, GC is the most widely used technique for determining sulfur compounds in wines and other matrices. Table 4 shows a selection features of chromatographic capillary columns, temperature programs and detection systems used in the analysis of these analytes in wines.

3.2.1. Injection systems

After concentrating the analytes, the sample is injected in the GC system in split or splitless mode, depending on the volume and concentration. Since many techniques are used to analyse sulfur compounds, different injection systems are often required.

When a PT concentrator is used for sampling, analytes are introduced into the chromatographic column through a transfer line after the desorption step because the injector is connected on-line to the concentrator system.

The static headspace technique involves injection of large volumes of gas so, a cryogenic trap helps to obtain better resolution [26,76,87]. Furthermore, there are special liners, which improve the response for this kind of injection.

Finally, when a sample is extracted with SPME, the diameter of the GC injection liner has an important influence on the peak shape, so an inlet liner of 0.75 mm I.D. has to be used [85,89].

3.2.2. Chromatographic columns

Several chromatographic columns, coated with different stationary phases, have been used in analysis of the sulfur compounds present in wines, depending on the polarity of the chemical structures to be assayed. Fused-silica capillary columns (Table 4) are mostly used. They are coated with polyethylene glycol (polar columns) or dimethyl polysiloxane (apolar columns). Some studies however report the use of packed columns, coated with DC 200 [40,54,78–80].

3.2.3. Detection systems

Highly sensitive and specific detectors are required to evaluate the low concentrations of the analytes. Two of the most important specific detection systems are flame photometric detection (FPD) and sulfur chemiluminescence detection (SCD).

The FPD response to sulfur-containing compounds is exponential and is dependent on both concentration and structure of the compound. A loss of sensitivity, caused by decreased emission, may however be observed with oxygen-containing compounds. In addition, the sensitivity of the detector may be affected when compounds with a considerable amount of carbon are monitored giving rise to an increase in the flame temperature (quenching) [40]. Despite these problems, the good sensitivity and the low cost of FPD make this detection method widely used in analysis of these compounds in wines [28,40,42,62,72,76,85–88].

An alternative to FPD is SCD. This detection method provides a linear and nearly equimolecular response to sulfur which is more sensitive than FPD. In addition, there are no problems of hydrocarbon quenching. However, it is critically dependent on such operating variables as the alignment of the probe or the temperature of flame ionisation detection–SCD transfer line [90]. In spite of these disadvantages, SCD is increasingly used for the analysis of sulfur compounds in wines [73,74,81,91,92].

Finally, the coupling of gas chromatography and mass spectrometry (GC-MS) was found to be a powerful tool in identification of new sulfur compounds [23,42,62,63,66,68,93], even if the system may not be considered a sulfur specific detector.

4. Conclusions

The literature describes several analytical methods for analysing sulfur compounds in wines, mostly involving the gas chromatographic technique. How-

Table 4
Chromatographic conditions in the analysis of sulfur compunds in wines[a]

Capillary column size	Temperature program	Detection	Ref.
· Innowax			
50 m×0.2 mm I.D.×0.2 μm	35°C (8 m), 50°C/m, 220°C (10 m)	FPD	[75–76]
	35°C (8 m), 50°C/m, 220°C (10 m)	FPD	[85]
	40°C (5 m), 3°C/m, 130°C, 40°C/m, 220°C, 10 m	FPD	[88]
· Carbowax 20 M			
50 m×0.32 mm I.D.×0.25 μm	60°C, 3°C/m, 220°C (35 m)	SCD	[83]
50 m×0.25 mm I.D.×0.2 μm	60°C, 3°C/m, 200°C (20 m)	MSD	[93,105]
60 m×0.2 mm (quartz column)	50°C, 2°C/m, 150°C	MSD	[66,67]
60 m×0,25 mm I.D.×0.25 μm	60°C (1 m), 2°C/m, 170°C	FPD	[42]
25 m 0.25 mm I.D.×1 μm	35°C (1 m), 3°C/m, 230°C (30 m)	MSD	[30,31]
	35°C (10 m), 3°C/m, 230°C (30 m)	MSD	[66]
· Carbowax DBWax(JW)			
30 m×0.32 mm I.D.×0.2 μm	40°C (5 m), 3°C/m, 200°C (20 m)	FPD/MSD	[93]
	40°C (5 m), 3°C/m, 200°C (20 m)	FPD/FID/MS	[41,105]
	40°C (5 m), 3°C/m, 200°C (20 m)	FPD/NPD/FID MSD/GCO	[29,69]
· CPWAX 52CB, Chrompack			
50 m×0.22 mm I.D.×0.25 μm	35°C (1 m), 3°C/m, 230°C (25 m)	FPD	[40,62,68]
· BP20 (SGE)			
50 m×0.25 mm I.D.×0.25 μm	35°C (30 m), 3°C/m, 230°C (30 m)	FPD	[42]
	35°C (1 m), 3°C/m, 230°C (15 m)	FPD/MSD	[28,72]
	35°C (1 m), 3°C/m, 230°C (15 m)	FPD/MSD/GCO	[71]
	35°C (1 m), 3°C/m, 230°C (25 m)	MSD	[62]
· BP X35			
50 m×0.22 mm I.D.×0.25 μm	35°C (1 m), 3°C/m, 230°C (15 m)	FPD/MSD/GCO	[32]
· BPX5			
50 m×0.22 mm I.D.×0.25 μm	35°C (1 m), 3°C/m, 230°C (15 m)	FPD/MSD/GCO	[32,71]
· SPB1			
30 m×0.32 mm I.D.×4 μm	35°C (20 m), 3°C/m, 230°C (30 m)	FPD	[42]
	35°C (1 m), 10°C/m, 230°C	SCD	[74]
	35°C (5 m), 10°C/m, 180°C (8 m)	SCD	[81]
	35°C (5 m), 10°C/m, 180°C (8 m)	SCD	[82]
	35°C (8 m), 15°C/m, 150°C, 40°C/m, 280°C (5 m)	FPD	[87]
	50°C (8 m), 15°C/m, 150°C, 40°C/m, 280°C (5 m)	FPD	[86]
	40°C, 10°C/m, 300°C (10 m)	SCD	[70]
· HP1			
wide bore 30 m×0.53 mm I.D.×0.88 μm	35°C (4 m), 30°C/m, 230°C	FPD	[26]

[a] REF.: Bibliographic references; FPD: flame photometric detector; SCD: sulfur chemiluminiscence detector; FID: flame ionisation detector; MSD: mass spectrometry detector; GCO: gas chromatography olfactometry.

ever, established methods have not paid enough attention to some relevant problems encountered in the determination of these compounds.

The first is inherent to a correct quantitative evaluation of the reference standards. In fact, several sulfur compounds, being volatile and/or oxidizable, demonstrated to be unstable and therefore difficult to quantify. In this case, the concentration of the

standards must be checked often, if necessary, daily. In addition, these standards should be handled carefully under a nitrogen atmosphere to avoid oxidation, and stored at low temperatures to avoid volatilisation losses. These precautions are generally not mentioned in the literature.

The second problem is related to the assessment of the methods developed. Many of them did not check the quality parameters that determine their suitability (detection and quantitation limits, recoveries and reproducibility). From analytical perspectives, these parameters are the essential bases necessary to validate any method and guarantee its application.

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References

[1] M.H. Boelens, L. van Gemert, Perfumer Flavorist 18 (1993) 29.
[2] J.A. Maga, CRC Crit. Rev. Food Sci. Nutr. 7 (1976) 147.
[3] C. Chen, S. Liu, C. Wu, C. Ho, in: T.H. Parliament, R. Croteau (Eds.), Biogeneration of Aromas, ACS Symposium Series, American Chemical Society, Washington, DC, 1986, p. 176, Chapter 14.
[4] R.C. Lindsay, J.K. Rippe, in: T.H. Parliament, R. Croteau (Eds.), Biogeneration of Aromas, ACS Symposium Series, American Chemical Society, Washington, DC, 1986, p. 286, Chapter 23.
[5] R. Artacho, M.F. Olea, M.D. Ruiz, Food Chem. 53 (1995) 91.
[6] M. Güntert, J. Brüning, R. Emberger, R. Hopp, M. Köpsel, H. Surburg, P. Werkhoff, in: R. Teranishi, G.R. Takeoka, M. Güntert (Eds.), Flavor Precursors, ACS Symposium Series, American Chemical Society, Washington, DC, 1992, p. 140, Chapter 11.
[7] K.B. de Roos, in: R. Teranishi, G.R. Takeoka, M. Güntert (Eds.), Flavor Precursors, ACS Symposium Series, American Chemical Society, Washington, DC, 1992, p. 203, Chapter 16.
[8] C.-Y. Tai, C.-T. Ho, J. Agric. Food Chem. 45 (1997) 3586.
[9] T. Hofmann, P. Schieberle, J. Agric. Food Chem. 45 (1997) 898.
[10] Y. Zheng, S. Brown, W. Ledig, C. Mussinan, C.-T. Ho, J. Agric. Food Chem. 45 (1997) 894.
[11] T. Hofmann, P. Schieberle, W. Grosch, J. Agric. Food Chem. 44 (1996) 251.
[12] P. Semmelroch, W. Grosch, J. Agric. Food Chem. 44 (1996) 537.
[13] D.C. Breeden, J.A. Juvik, J. Food Comp. Anal. 5 (1992) 134.
[14] A. Hinterholzer, P. Schieberle, Flavour Fragr. J. 13 (1998) 49.
[15] P. Werkhoff, M. Güntert, G. Krammer, H. Sommer, J. Kaulen, J. Agric. Food Chem. 46 (1998) 1076.
[16] F. Pelusio, T. Nilsson, L. Montanarella, R. Tilio, B. Larsen, S. Facchetti, J. Madsen, J. Agric. Food Chem. 43 (1995) 2138.
[17] S. Sakuma, V. Rikimaru, K. Obayashi, M. Kowaka, ASBC J. 49 (1991) 162.
[18] A. Dercksen, I. Meijering, B. Axcell, ASBC J. 50 (1992) 93.
[19] A. Irwin, L. Bordeleau, R.L. Barker, ASBC J. 51 (1993) 1.
[20] H. Goldstein, S. Rader, A. Murakami, ASBC J. 51 (1993) 70.
[21] A. Kamiya, Y. Ose, J. Chromatogr. 292 (1984) 383.
[22] O.J. Goniak, A.C. Noble, Am. J. Enol. Vitic. 38 (1987) 223.
[23] D. Rauhut, W. Sponholz, in: T. Henick-Kling (Ed.), Proceedings of the 7th Australian Wine Industry Technical Conference, Adelaide, 1990, p. 196.
[24] D. Rauhut, W. Sponholz, in: Proceedings of the ASEV/ES Workshop. Wine Aroma Defects, Corning, NY, 1992, p. 44.
[25] D. Rauhut, H. Kürbel, H.H. Dittrich, Vitic. Enol. Sci. 48 (1993) 214.
[26] S.K. Park, R.B. Boulton, E. Bartra, A.C. Noble, Am. J. Enol. Vitic. 45 (1994) 341.
[27] T. Tominaga, P. Darriet, D. Dubourdieu, Vitis 35 (1996) 207.
[28] P. Bouchilloux, Ph. Darriet, D. Dubourdieu, J. Int. Sci. Vigne Vin 30 (1996) 23.
[29] P. Guedes de Pinho, A. Anocibar Beloqui, A. Bertrand, Sci. Aliments 17 (1997) 341.
[30] T. Tominaga, A. Furrer, R. Henry, D. Dubourdieu, Flavour Fragr. J. 13 (1998) 159.
[31] T. Tominaga, M.L. Murat, D. Dubourdieu, J. Agric. Food Chem. 46 (1998) 1044.
[32] P. Bouchilloux, P. Darriet, R. Henry, V. Lavigne, D. Dubourdieu, J. Agric. Food Chem. 46 (1998) 3095.
[33] P. Schreier, CRC Crit. Rev. Food Sci. Nutr. 12 (1979) 59.
[34] A. Rapp, H. Mandery, Experientia 42 (1986) 873.
[35] H. Maarse, C.A. Visscher (Eds.), Volatile Compounds in Food – Alcoholic Beverages, TNO-CIVO, Zeist, 1989, p. 109.
[36] P.X. Etiévant, in: H. Maarse (Ed.), Volatile Compounds in Foods and Beverages, Marcel Dekker, New York, 1991, p. 483.
[37] V. Ferreira, J. Cacho, A. Escudero, C. Peña, A. Lopez, P. Fernandez, in: XX Congreso Mundial de la Viña y el Vino. 72 Asamblea General de la OIV, Madrid y La Rioja, 1992, Vol. III, Section 2.
[38] P. Dubois, Rev. Franç. Oenolog. 146 (1994) 39.
[39] H. Guth, J. Agric. Food Chem. 45 (1997) 3022.
[40] P. Chatonnet, in: B. Doneche (Ed.), Les Adquisitions Récentes en Chromatographie du Vin – Applications à l'Analyse Sensorielle des Vins, Tec & Doc. Lavoisier, Paris, 1993, p. 121.
[41] A. Anocibar Beloqui, A. Bertrand, Ital. J. Food Sci. 3 (1995) 279.

[42] P. Darriet, T. Tominaga, V. Lavigne, J.N. Boidron, D. Dubourdieu, Flavour Fragr. J. 10 (1995) 385.
[43] G.J. Loubser, C.S. Du Plessis, Vitis 15 (1976) 248.
[44] R.F. Simpson, Vitis 18 (1979) 148.
[45] D.J. Spedding, R. Eschenbruch, A. Purdie, Vitis 19 (1980) 241.
[46] D.J. Spedding, P. Raut, Vitis 21 (1982) 240.
[47] A. Maujean, Rev. Oenolog. 53S (1989) 31.
[48] D.J. Spedding, P. Raut, Vitis 21 (1982) 240.
[49] E. Minarik, Bull. OIV 559 (1977) 640.
[50] R. Eschenbruch, S.J. de Mora, S.J. Knowles, W.K. Leonard, T. Forrester, D.J. Spedding, Vitis 25 (1986) 53.
[51] B.W. Zoecklein, K.C. Fugelsang, B.H. Gump, F.S. Nury, in: Production Wine Analysis, Van Nostrand Reinhold, New York, 1990, p. 207, Chapter 10.
[52] V. Lavigne, D. Dubourdieu, Rev. Oenolog. 85 (1997) 23.
[53] A. Maujean, M. Haye, M. Feuillat, Connaiss. Vigne Vin 12 (1978) 277.
[54] A. Maujean, N. Seguin, Sci. Aliment. 3 (1983) 589.
[55] V. Lavigne, D. Dubourdieu, in: Proceedings of the 5th Symposium International d'Oenologie: Oenologie '95, Bordeaux, 1995, p. 251.
[56] T. Tominaga, I. Masneuf, D. Dubourdieu, J. Int. Sci. Vigne Vin 29 (1995) 227.
[57] T. Tominaga, C. Peyrot, D. Dubourdieu, J. Agric. Food Chem. 46 (1998) 5215.
[58] C.S. Thomas, R. Boulton, M.W. Silacci, W.D. Gubler, Am. J. Enol. Vitic. 44 (1993) 211.
[59] S.K. Park, A.C. Noble, in: Conaissance Aromatique des Cépages et Qualité des Vins – Actes du Symposium International, 1993, p. 328, Revue Française d'OEnologie (Eds.).
[60] M. Schütz, R.E. Kunkee, Am. J. Enol. Vitic. 28 (1997) 137.
[61] B. Haye, A. Maujean, C. Jacquemin, M. Feuillat, Connaiss. Vigne Vin 11 (1977) 243.
[62] V. Lavigne, R. Henry, D. Dubourdieu, Sci. Aliment. 18 (1998) 175.
[63] C.J. Muller, R.E. Kepner, A. Dinsmoor Webb, Am. J. Enol. Vitic. 22 (1971) 156.
[64] P. Schreier, F. Drawert, A. Junker, Z. Lebensm. Unters. Forsch. 154 (1974) 279.
[65] P. Schreier, F. Drawert, Z. Lebensm. Unters. Forsch. 154 (1974) 27.
[66] A. Rapp, M. Güntert, J. Almy, Vitis 23 (1984) 66.
[67] A. Rapp, M. Güntert, J. Almy, Am. J. Enol. Vitic. 36 (1985) 219.
[68] P. Chatonnet, V. Lavigne, J.N. Boidron, D. Dubourdieu, Sci. Aliment. 12 (1992) 513.
[69] A. Anocibar Beloqui, P. Guedes, A. Bertrand, J. Int. Vigne Vin 29 (1995) 17.
[70] A. Lavigne-Delcroix, D. Tusseau, M. Proix, Sci. Aliment. 16 (1996) 267.
[71] T. Tominaga, P. Darriet, D. Dubourdieu, Vitis 35 (1996) 207.
[72] P. Darriet, P. Bouchilloux, V. Lavigne, R. Henry, D. Dubourdieu, T. Tominaga, in: First Symposium Proceedings of the In Vino Analytica Scientia, Bordeaux, 12–14 June 1997, p. 50.
[73] M. Nedjma, A. Maujean, J. Chromatogr. A 704 (1995) 495.
[74] J.P. Vidal, S. Estreguil, G. Snakkers, R. Cantagrel, in: Proceedings of the 5th Symposium International d'Oenologie: Oenologie '95, Bordeaux, 1995, p. 587.
[75] M. Mestres, O. Busto, J. Guasch, in: First Symposium Proceedings of the In Vino Analytica Scientia, Bordeaux, 12–14 June 1997, p. 325.
[76] M. Mestres, O. Busto, J. Guasch, J. Chromatogr. A 773 (1997) 261.
[77] A. Maujean, M. Nedjma, J.P. Vidal, R. Cantagrel, L. Lurton, in: R. Cantagrel (Ed.), Elaboration et Conaissance des Spiritueux, Lavoisier Tec. & Doc., 1993, p. 110.
[78] V. Lavigne, J.N. Boidron, D. Dubourdieu, J. Int. Sci. Vigne Vin 27 (1993) 1.
[79] O. Leppänen, J. Denslow, P. Ronkainen, J. Inst. Brew. 85 (1979) 350.
[80] O. Leppänen, J. Denslow, P. Ronkainen, J. Agric. Food Chem. 28 (1980) 359.
[81] D. Rauhut, H. Kürbel, K. McNamara, M. Grossmann, in: First Symposium Proceedings of the In Vino Analytica Scientia, Bordeaux, 12–14 June 1997, p. 169.
[82] D. Rauhut, H. Kürbel, K. McNamara, M. Grossmann, Analusis 26 (1998) 142.
[83] D. Rauhut, H. Kürbel, Vitic. Enol. Sci. 49 (1994) 27.
[84] N. Gandini, R. Riguzzi, J. Agric. Food Chem. 45 (1997) 3092.
[85] M. Mestres, O. Busto, J. Guasch, J. Chromatogr. A 808 (1998) 211.
[86] M. Mestres, C. Sala, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A 835 (1999) 137.
[87] M. Mestres, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A 849 (1999) 293.
[88] M. Mestres, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A. (2000) in press.
[89] X. Yang, T. Peppard, J. Agric. Food Chem. 42 (1994) 1925.
[90] A.W. Dercksen, I. Meijering, B. Axcell, ASBC J. 50 (1992) 93.
[91] D. Rauhut, H. Kürbel, in: Proceedings of the 5th Symposium International d'Oenologie: Oenologie '95, Bordeaux, 1995, p. 515.
[92] M. Nedjma, A. Maujean, in: Proceedings of the 5th Symposium International d'Oenologie: Oenologie '95, Bordeaux, 1995, p. 583.
[93] A. Anocibar Beloqui, P. Guedes de Pinho, A. Bertrand, Am. J. Enol. Vitic. 46 (1995) 84.
[94] J.J. Descout, Rev. Oenolog. Tech. 65 (1995) 15.
[95] D.J. Spedding, R. Eschenbruch, P.J. McGregor, Food Technol. Australia 35 (1983) 22.
[96] G. Montedoro, M. Bertuccioli, in: I.D. Morton, A.J. Mcleod (Eds.), Development in Food Sciences, 3B, Food Flavours, Part B, The Flavour of Beverages, Elsevier Science Amsterdam, 1986, p. 171, Chapter V.
[97] A. Rapp, in: Les Acquisitions Récentes en Chromatographie du Vin, Lavoisier, Porto, 1992, p. 151.
[98] J. Marais, Vitis 18 (1979) 254.
[99] S.-J. de Mora, S.J. Knowles, R. Eschenbruch, W.J. Torrey, Vitis 26 (1987) 79.
[100] Y. Le Fur, M. Feuillat, P.X. Étievant, Rev. Fr. Oenolog. 128 (1991) 7.

[101] J.D. Dubourdieu, V. Lavigne, in: INFRE-VITI'92, Bordeaux, 1992, p. 41.
[102] S.J. de Mora, P. Lee, D. Shooter, R. Eschenbruch, Am. J. Enol. Vitic. 44 (1993) 327.
[103] V. Lavigne, J.N. Boidron, D. Dubourdieu, J. Int. Sci. Vigne Vin 26 (1992) 75.
[104] P. Guedes, A. Bertrand, in: Proceedings of the 5th Symposium International d'Oenologie: Oenologie '95, Bordeaux, 1995, p. 549.
[105] A. Anocibar Beloqui, A. Bertrand, in: Proceedings of the 5th Symposium International d'Oenologie: Oenologie '95, Bordeaux, 1995, p. 593.
[106] T. Herraiz, G. Reglero, P.J. Martín-Alvarez, M. Herraiz, M.D. Cabezudo, J. Sci. Food Agric. 55 (1991) 103.
[107] D. Dubourdieu, P. Darriet, J. Vignevini 7–8 (1993) 38.
[108] J. Marais, S. Afr. J. Enol. Vitic. 15 (1994) 41.
[109] D. Dubourdieu, Rev. Oenolog. Tech. 79 (1997) 18.
[110] D. Rauhut, in: P. Ribereau-Gayon, A. Lonvaud (Eds.), Actualités Oenologiques 89, Dunod, 1989, p. 482.
[111] H. Guth, J. Agric. Food Chem. 45 (1997) 3027.