

Volatile sulphur compounds composition of monovarietal white wines

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A B S T R A C T

The sulphur compounds composition of wines produced experimentally from six white cultivars (Alvarinho, Loureiro, Trajadura, Pedernã, Azal Branco and Avesso) was evaluated during two consecutive vintages. Results show that wines could be differentiated according to their sulphur compounds content. In general, Loureiro, Trajadura and Pedernã cultivars led to wines with low concentrations of sulphur compounds; however, Loureiro wines were characterised by significant amounts of dimethyl sulphone, whereas Trajadura wines possessed a high content of 3-(methylthio)propyl acetate and 4-(methylthio)-1-butanol. Alvarinho and Avesso wines showed high levels of S-methyl thioacetate, 3-mercapto-1-propanol, 3-(ethylthio)-1-propanol and 3-methylthiopropionic acid. Significant amounts of 2-methyl-tetrahydrothiophen-3-one, *cis*- and *trans*-2-methyltetrahydrothiophen-3-ol were also found in Avesso wines. Azal Branco wines were low in 3-(methylthio)propyl acetate and 2-(methylthio)ethanol, and high contents in S-methyl thioacetate, 3-mercapto-1-propanol and 2-mercaptoethanol. A linear discriminant analysis of sulphur compounds levels showed a differentiation of wines according to their varietal origin.

Introduction

Thiols, disulphides and other sulphur-containing compounds play an important role in wine aroma. Sulphur compounds contribute mainly to unpleasant aromas in wines, although some of them have been reported to have a positive contribution to wine, mainly 4-mercapto-4-methyl-2-pentan-one, 3-mercaptohexyl acetate, 3-mercapto-1-hexan-ol, 4-mercapto-4-methyl-2-pentan-ol and 3-mercapto-3-methyl-1-butan-ol (Bouchilloux, Darriet, Henry, Lavigne-Cruège, & Dubourdieu, 1998; Sarrazin et al., 2007; Tominaga & Dubourdieu, 2006; Vermeulen, Gijs, & Collin, 2005). Intensity and sensory impression of off-flavours depend on the qualitative and quantitative composition of volatile sulphur compounds in wines (Rauhut & Kürbel, 1995). The light sulphur compounds (hydrogen sulphide, ethanethiol and methanethiol), possessing high volatility and low perception values, can be eliminated by simple racking or aeration, and by copper treatment (Anocibar Beloqui & Bertrand, 1995; Darriet, Lavigne-Cruège, & Tominaga, 1999; Karagiannis & Lanaridis, 2000). The concentration of these compounds in reduced wines is higher than their perception thresholds and always higher

than their concentrations in wines without off-flavours (Darriet et al., 1999; Mestres, Busto, & Guasch, 2002). The heavy sulphur compounds cannot be eliminated and may have an unpleasant aroma even at low concentrations; moreover, they can become precursors of light sulphur compounds during wine storage (Anocibar Beloqui & Bertrand, 1995; Mestres, Busto, & Guasch, 2000). Threshold values and odour notes of heavy sulphur compounds are described in Table 1.

The volatile sulphur compounds in wines come mainly from the metabolism of yeast. The mechanisms of production of these compounds by yeasts are various and complex. The yeast strain, the grape variety, the ampelographic origin of wines, the vinification conditions, and the redox potential of must play a fundamental role on the levels of some sulphur compounds in wines (Falqué, Fernández, & Dubourdieu, 2002; Spiropoulos, Tanaka, Flerianos, & Bisson, 2000; Thomas, Boulton, Silacci, & Gubler, 1993). A high turbidity of grape juice, a low nitrogen source and pantothenate deficiency of musts, a high temperature of fermentation and the excessive addition of sulphur dioxide to grape musts lead to an increase of sulphur compounds in wines; the conditions of conservation of wines in barriques or in stainless tanks, the duration of contact of wines with lees, and the storage time may also have important effects on the formation of these compounds in wines (Fedrizzi, Magno, Badocco, Nicolini, & Versini, 2007; Karagiannis

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Table 1
Odour/flavour notes and threshold values of heavy sulphur compounds.

Sulphur compound	Odour/flavour notes	Threshold values
Methionol	Cooked potato, cauliflower, cooked vegetable/cabbage ^{a,b,c,d,e,f}	3200 µg/l in red wine, 4500 µg/l in white wine ^{b,d,g} ; 500 µg/l in wine ^{f,h}
S-Methyl thioacetate	Rotten/cooked vegetables, sulphurous ^{e,f}	–
3-Mercapto-1-propanol	Sweat odour, roasted, potato, broth ^{d,i}	60 µg/l in model solution ^d
2-Mercaptoethanol	Box tree, poultry, farmyard, alliaceous, burnt rubber ^{c,e,j,k}	1–10 mg/l in hydroalcoholic solution ⁱ ; 600 µg/l in red wine, 450 µg/l in white wine ^d ; 0.13–10 mg/l in wine ^e
Dimethyl sulphone	Odourless ^l	–
N-3-(Methylthiopropyl)acetamide	Odourless ^e	–
3-(Methylthio)propyl acetate	Mushroom, onion, garlic ^{b,c,d,e,g,k}	115 µg/l in red wine, 100 µg/l in white wine ^{b,d,g}
4-(Methylthio)-1-butanol	Metallic-bitter, grassy, onion, earthy, chive-garlic ^{b,d,e,j,k}	80 µg/l in wine ^k ; 0.1–1.0 mg/l in hydroalcoholic solution ⁱ ; 0.1 mg/l in wine ^e
3-(Ethylthio)-1-propanol	Rancid, sweaty ^a	–
2-(Methylthio)ethanol	French bean, cauliflower ^{c,d,e,k}	250 µg/l in wine ^k ; 640 µg/l in red wine, 800 µg/l in white wine ^d
3-Methylthiopropionic acid	Chocolate, roasted, butter, rancid ^{d,e,m}	50 µg/l in model solution ^d ; 244 µg/l in red wine ^m
2-Methyltetrahydrothiophen-3-one	Metallic, natural gas, butane-like ^{c,d,e,k}	250 µg/l in red wine, 150 µg/l in white wine ^d
cis-2-Methyltetrahydrothiophen-3-ol	Odourless ^{b,d,e}	–
trans-2-Methyltetrahydrothiophen-3-ol	Onion, chive-garlic ^{b,c,d,k}	100–500 µg/l in hydroalcoholic solution ^j

^a Baumes et al. (1986).

^b Chatonnet et al. (1992).

^c Anocibar Beloqui and Bertrand (1995).

^d Lavigne (1996).

^e Mestres et al. (2000).

^f Landaud, Helinck, and Bonnarme (2008).

^g Lavigne et al. (1992).

^h Swiegers, Bartowsky, Henschke, and Pretorius (2005).

ⁱ Vermeulen et al. (2005).

^j Rapp, Güntert, and Almy (1985).

^k Darriet et al. (1999).

^l Ferreira, Rodrigues, Hogg, and Guedes de Pinho (2003).

^m Pripis-Nicolau, Revel, Bertrand, and Lonvaud-Funel (2004).

& Lanaridis, 1999, 2000; Lavigne & Dubourdiou, 1996; Park, Boulton, & Noble, 2000; Vasserot, Steinmetz, & Jeandet, 2003; Wang, Bohlscheid, & Edwards, 2003).

The *Vinhos Verdes* Denomination of Origin is located in the North-west region of Portugal. The white wines possess an alcoholic content between 8.5% and 11.5% (v/v), except the Alvarinho variety, whose alcoholic content must be between 11.5% and 13%. These wines are slightly acidic and characterised by some effervescence. They are classified as soft wines with fruity notes of medium to high intensity. Very few reports are available in the literature concerning the volatile characterisation of white grape musts and wines from the Alvarinho, Loureiro, Trajadura, Pedernã, Azal Branco and Avesso cultivars; measurements of phenolic compounds, alcohols, organic acids and monoterpenic compounds composition of grapes and wines have been reported (Dopico-García, Valentão, Guerra, Andrade, & Seabra, 2007; Oliveira et al., 2004; Oliveira, Faria, Sá, Barros, & Araújo, 2006). The aim of this study is to evaluate the heavy sulphur compounds composition of *Vinhos Verdes* and investigate if cultivars can be differentiated according to that composition.

Materials and methods

Grape musts and vinification conditions

Experiments were carried out with white musts obtained from grapes of the *Vitis vinifera* varieties of the *Vinhos Verdes* Region: Alvarinho, Loureiro, Trajadura, Pedernã, Azal Branco and Avesso. Each variety was studied during two consecutive vintages.

The same technological procedure was applied in the production of all wines. Grapes were picked at random, from the same location. Harvested grapes were crushed, pressed and treated with sulphite solution to achieve 30 mg/l. After settling overnight at 5 °C, grape musts were racked into 10-l glass tanks. All experiments were carried out in duplicate. Fermentations were initiated by starter cultures of rehydrated *Saccharomyces cerevisiae* at 20

g/hl (commercial name Fermol Bouquet, from Pascal Biotech, controlled by the General Microbiology Laboratory of the Faculty of Science of the University of Reims-Champagne, France). Fermentations were carried out at 18 °C and considered complete when no variation of sugar content was observed (below 5 g/l), and before occurrence of malolactic fermentation. Wines were cold-stabilized and then the sulphite content was adjusted to 30 mg/l free. After bottling, wines were stored at 15 °C.

Analytical determinations

For each wine, the total and volatile acidity, pH, alcoholic degree, free and total SO₂ were determined using standard procedures, as described by the Office International de la Vigne et du Vin (OIV, 1990).

Sulphur compounds were determined by gas chromatography with flame photometric detection (GC/FPD), according to the method described by Moreira, Guedes de Pinho, and Vasconcelos (2004). The sulphur compounds analysed were (CAS number in brackets): S-methyl thioacetate [1534-08-3], 2-mercaptoethanol [60-24-2], 2-(methylthio)ethanol [5271-38-5], 2-methyltetrahydrothiophen-3-one [13679-85-1], 3-(methylthio)propyl acetate [16630-55-0], 3-methylthiopropionic acid [646-01-05], 3-mercapto-1-propanol [19721-22-3], 3-(methylthio)-1-propanol (methionol) [505-10-2], *cis*- and *trans*-2-methyltetrahydrothiophen-3-ol ([62614-75-9] and [62614-77-1], respectively), 3-(ethylthio)-1-propanol [18721-61-4], 4-(methylthio)-1-butanol [20582-85-8], dimethyl sulphone [67-71-0] and *N*-[3-(methylthio)propyl]acetamide [54824-91-8]. For those compounds whose reference standards were commercially available, a calibration per mass was carried out and concentrations were expressed as µg/l. Amounts of compounds whose reference standards were not commercially available were expressed as:

peak area × 1000/peak area of internal standard.

Statistical analysis

An analysis of variance (ANOVA) was applied to the experimental data; results were considered significant if the associated *p* value was below 0.05. The significant differences were determined by Tukey tests. A principal component analysis and a linear discriminant analysis were also applied to data. All statistical analysis were performed using SPSS for Windows, Version 10.0 (SPSS Inc., Chicago, IL).

Results and discussion

Wine parameters

Significant differences of pH, total acidity, alcoholic degree and total SO₂ were obtained in wines resulting from different grape cultivars (Table 2). The pH values varied between 2.19 and 3.13, and presented the highest values in Year 2, except for Loureiro and Trajadura wines. The highest amounts of total acidity were found in Azal Branco wines in both vintages (11.7 and 10.9 g/l), as well as in Loureiro wines in the 2nd year (11.0 g/l). The Alvarinho and Trajadura wines presented the lowest total acidity values (7.3–8.1 g/l) in both vintages. The values of volatile acidity were similar in all wines, varying between 0.34 and 0.65 g/l. The highest alcoholic degree values were obtained, as expected, in Alvarinho wines (11.6% and 12.4%); this parameter varied between 8.88%

and 10.9% for the other wines. These values are in agreement with Portuguese legislation for the Appellation of Origin *Vinhos Verdes* that recommends/imposes an alcoholic degree between 8.0% and 11.5%, except for Alvarinho wines, whose values must be between 11.5% and 13.0%, and a total acidity of at least 6.0 g/l (4.5 g/l for Alvarinho wine). Previously published data for the same grape cultivars presented similar values of pH, varying between 2.70 and 3.08, total acidity, within 6.57 and 11.4 g/l, volatile acidity, within 0.24 and 0.81 g/l, and alcoholic degree, varying between 8.9% and 12.8%; Alvarinho showed higher alcoholic degree values, 13.5–13.9% (Oliveira et al., 2006).

The free SO₂ concentration presented similar values in wines of Year 2; however, for Year 1, some differences were observed, with Trajadura, Pedernã and Azal Branco wines presenting the lowest contents of free SO₂. The total SO₂ concentration in wines also varied, and the highest values in both vintages were observed in Trajadura wines.

Principal component analysis of data

Sulphur compounds content of wines are presented in Tables 3 and 4. In general, when significant, the amounts of sulphur compounds in wines were higher in Year 2 than in Year 1.

A principal component analysis (PCA) was applied to all samples to obtain a simplified view of the relationship between the wines. Three factors explain 72.8% of the total variance. The

Table 2
Oenological parameters of Trajadura, Pedernã, Loureiro, Alvarinho, Azal Branco and Avesso wines.

Vintage	Parameters	Trajadura	Pedernã	Loureiro	Alvarinho	Azal Branco	Avesso
Year 1	pH	2.96 (0.02) ^b	2.72 (0.07) ^a	2.64 (0.03) ^a	2.70 (0.02) ^a	2.63 (0.05) ^a	2.67 (0.02) ^a
	Total acidity (g/l) ^A	7.4 (0.2) ^a	9.8 (0.5) ^c	8.8 (0.3) ^b	8.0 (0.3) ^a	11.7 (0.30) ^d	10.1 (0.2) ^c
	Volatile acidity (g/l) ^B	0.46 (0.03)	0.65 (0.18)	0.34 (0.04)	0.44 (0.07)	0.61 (0.16)	0.45 (0.05)
	Alcoholic degree (v/v)	9.13 (0.22) ^a	9.62 (0.99) ^a	9.64 (0.21) ^a	12.4 (0.5) ^c	8.88 (0.42) ^a	10.9 (0.1) ^b
	Free SO ₂ (mg/l)	11.8 (2.1) ^a	11.3 (1.5) ^a	20.5 (5.0) ^b	21.3 (2.22) ^b	15.0 (2.1) ^{ab}	21.3 (4.9) ^b
	Total SO ₂ (mg/l)	124 (3.4) ^b	92.0 (15.4) ^a	92.3 (14.8) ^a	80.5 (4.8) ^a	79.8 (2.7) ^a	73.8 (1.7) ^a
Year 2	pH	2.89 (0.13) ^b	2.98 (0.10) ^{bc}	2.19 (0.19) ^a	3.13 (0.04) ^c	2.88 (0.06) ^b	3.02 (0.13) ^{bc}
	Total acidity (g/l) ^A	7.3 (0.5) ^a	9.2 (0.7) ^c	11.0 (0.6) ^d	8.1 (0.3) ^b	10.9 (0.7) ^d	9.4 (0.5) ^c
	Volatile acidity (g/l) ^B	0.39 (0.09)	0.42 (0.06)	0.42 (0.11)	0.52 (0.16)	0.40 (0.05)	0.49 (0.06)
	Alcoholic degree (v/v)	9.56 (0.44) ^b	10.8 (0.2) ^c	8.89 (0.52) ^a	11.6 (0.3) ^d	9.92 (0.33) ^b	10.9 (0.4) ^c
	Free SO ₂ (mg/l)	23.9 (5.4)	29.8 (6.6)	27.5 (11.7)	24.9 (7.7)	23.9 (8.1)	29.0 (7.3)
	Total SO ₂ (mg/l)	105 (15) ^{bc}	89.1 (11.2) ^b	101 (19) ^{bc}	118 (25) ^c	64.3 (14.1) ^a	92.9 (18.9) ^b

Values in parenthesis are standard deviations from four determinations; values not sharing the same superscript letter (a, b, c, d) within the horizontal line are different according to the Tukey test.

^A Expressed as tartaric acid.

^B Expressed as acetic acid.

Table 3
Heavy sulphur compounds contents (µg/l) of Loureiro, Trajadura and Pedernã wines in two vintages.

Compound	Loureiro			Trajadura			Pedernã		
	Year 1	Year 2	Sig.	Year 1	Year 2	Sig.	Year 1	Year 2	Sig.
Methionol	412 (125)	611 (155)	ns	374 (1)	758 (292)	ns	390 (1)	1122 (168)	0.025
S-Methyl thioacetate	3.71 (0.01)	10.2 (2.0)	0.044	2.24 (0.34)	8.43 (0.35)	0.003	1.92 (0.12)	6.27 (1.46)	ns
3-Mercapto-1-propanol	20.0 (4.2)	19.5 (2.4)	ns	12.1 (0.1)	21.8 (3.9)	ns	9.87 (0.18)	28.4 (5.3)	0.038
2-Mercaptoethanol	30.2 (7.9)	25.2 (1.7)	ns	13.1 (0.2)	46.2 (1.8)	0.001	15.1 (0.1)	52.0 (7.7)	0.021
Dimethyl sulphone	42.2 (0.7)	38.3 (0.9)	0.042	13.8 (1.2)	18.2 (4.0)	ns	12.2 (0.3)	24.0 (1.9)	0.013
3-(Methylthio)propyl acetate ^a	1.40 (0.28)	8.60 (0.01)	0.001	5.10 (0.14)	25.8 (4.5)	0.023	2.75 (0.35)	28.9 (3.8)	0.011
4-(Methylthio)-1-butanol	14.6 (1.0)	20.6 (3.9)	ns	25.4 (0.6)	28.7 (6.0)	ns	11.6 (2.2)	26.1 (0.8)	0.013
N-3-(Methylthiopropyl)acetamide ^a	390 (73)	278 (66)	ns	30.6 (0.6)	1119 (77)	0.002	22.1 (0.1)	656 (56)	0.004
3-(Ethylthio)-1-propanol	21.4 (1.1)	16.5 (1.1)	0.048	19.0 (0.1)	21.8 (2.4)	ns	9.33 (0.46)	18.2 (1.6)	0.017
3-Methylthiopropionic acid ^a	82.1 (11.0)	75.1 (6.2)	ns	16.2 (0.2)	170 (13)	0.004	16.1 (0.1)	230 (4)	0.000
2-Methyltetrahydrothiophen-3-one	64.4 (12.5)	111 (2)	0.034	70.3 (0.4)	221 (76)	ns	85.0 (1.4)	134 (19)	ns
cis-2-Methyltetrahydrothiophen-3-ol ^a	20.4 (1.9)	92.4 (18.8)	0.033	61.5 (0.7)	194 (56)	ns	62.7 (0.4)	106 (1)	0.000
trans-2-Methyltetrahydrothiophen-3-ol ^a	12.4 (0.6)	123 (2)	0.000	71.1 (1.3)	231 (50)	ns	54.2 (0.2)	129 (3)	0.001
2-(Methylthio)ethanol	7.51 (0.27)	8.61 (0.94)	ns	11.8 (0.3)	11.5 (0.6)	ns	10.8 (0.3)	18.3 (1.0)	0.010

Values in parenthesis are standard deviations from four determinations. Sig.: significance; ns: not significant (*p* > 0.05).

^a Peak area × 10³ per peak area of internal standard.

Table 4Heavy sulphur compounds contents ($\mu\text{g/l}$) of Alvarinho, Azal Branco and Avesso wines in two vintages.

Compound	Alvarinho			Azal Branco			Avesso		
	Year 1	Year 2	Sig.	Year 1	Year 2	Sig.	Year 1	Year 2	Sig.
Methionol	465 (28)	690 (50)	0.032	384 (49)	857 (7)	0.006	472 (3)	898 (126)	0.041
S-Methyl thioacetate	8.46 (0.45)	11.5 (7.6)	ns	8.34 (2.14)	13.4 (0.5)	ns	8.52 (0.18)	13.6 (0.7)	0.011
3-Mercapto-1-propanol	19.9 (1.3)	62.0 (13.2)	0.047	26.6 (2.5)	44.9 (3.2)	0.024	38.5 (9.2)	37.9 (0.6)	ns
2-Mercaptoethanol	30.1 (13.7)	37.6 (21.6)	ns	57.9 (10.2)	19.0 (0.6)	0.033	71.5 (1.6)	57.2 (6.6)	ns
Dimethyl sulphone	23.8 (3.8)	15.1 (0.3)	ns	14.3 (0.4)	30.3 (1.4)	0.004	16.7 (1.7)	28.7 (1.5)	0.017
3-(Methylthio)propyl acetate ^a	1.60 (0.57)	6.92 (1.24)	0.032	1.09 (0.08)	9.25 (1.5)	0.017	2.75 (0.49)	19.0 (1.1)	0.003
4-(Methylthio)-1-butanol	10.9 (1.5)	19.1 (0.7)	0.019	21.0 (1.4)	15.9 (3.1)	ns	9.85 (0.81)	11.1 (2.3)	ns
N-3-(Methylthiopropyl)acetamide ^a	32.9 (3.7)	20.0 (1.1)	0.040	72.5 (19.1)	29.1 (2.2)	ns	40.8 (1.9)	193 (35)	0.025
3-(Ethylthio)-1-propanol	23.0 (8.5)	61.0 (13.2)	ns	21.8 (4.4)	22.8 (3.1)	ns	30.8 (1.3)	16.3 (0.9)	0.006
3-Methylthiopropionic acid ^a	176 (53)	683 (318)	ns	24.3 (2.3)	301 (22)	0.003	256 (54)	199 (1)	ns
2-Methyltetrahydrothiophen-3-one	42.6 (7.4)	138 (29)	0.046	97.8 (0.5)	136 (10)	0.034	245 (84)	155 (13)	ns
cis-2-Methyltetrahydrothiophen-3-ol ^a	58.0 (7.1)	224 (9)	0.002	31.4 (6.3)	80.0 (16.0)	ns	243 (124)	144 (21)	ns
trans-2-Methyltetrahydrothiophen-3-ol ^a	19.2 (2.1)	139 (5)	0.001	21.6 (2.1)	82.5 (10.4)	0.015	303 (136)	243 (28)	ns
2-(Methylthio)ethanol	10.6 (1.7)	15.0 (0.9)	ns	6.70 (0.14)	8.72 (0.60)	0.043	6.20 (0.14)	13.3 (0.8)	0.007

Values in parenthesis are standard deviations from four determinations. Sig.: significance; ns: not significant ($p > 0.05$).

^a Peak area $\times 10^3$ per peak area of internal standard.

representation of the variables and samples using the first two PCs is shown in Fig. 1A and B. The variables 4-(methylthio)-1-butanol, dimethyl sulphone, volatile acidity, free and total SO_2 were not used in the analysis, as they presented a communality value lower than 0.5. The variables with higher contribution to the first principal component (PC1), which explains 39.4% of total variance, are the alcoholic degree (alc), 3-mercapto-1-propanol (S6), 2-methyltetrahydrothiophen-3-one (S4), 3-methylthiopropionic acid (S9), *cis*- (S11) and *trans*-2-methyltetrahydrothiophen-3-ol (S12). All these variables are positively correlated to PC1. The second principal component (PC2), which explains 17.6% of total variance, is positively related to total acidity (AcT), and negatively related to 2-(methylthio)ethanol (S3), 3-(methylthio)propyl acetate (S5) and *N*-3-(methylthiopropyl)acetamide (S10). Results show that wines tend to separate according to the vintage, except for Loureiro wines. Trajadura, Pedernã, Alvarinho and Azal Branco wines from the 1st vintage and all Loureiro wines are placed in the negative PC1, being characterised by lower contents of the associated variables, whereas wines from the 2nd vintage, and all Avesso wines, are mainly related to the positive PC1. Trajadura and Pedernã wines of the same vintage, placed closer in the projection, are similar concerning the variables used, and are related to the negative PC2. The differences observed according to the vintage may be associated to different climatic conditions, which affect the nutrients level of grape musts (Park et al., 2000; Wang et al., 2003).

Heavy sulphur compounds composition of wines

Methionol is the heavy sulphur compound present in wines in the highest concentrations (Tables 3 and 4). No significant differences were obtained in methionol contents of wines from the same vintage. In Year 1, wines presented concentrations of methionol varying from 374 to 472 $\mu\text{g/l}$, whereas higher values of this compound (611–1122 $\mu\text{g/l}$) were obtained in Year 2. These methionol concentrations were below its threshold value in white wines (Table 1). According to Falqué et al. (2002), methionol concentration was one of the variables responsible for the differentiation of wines from Loureiro, Dona Branca and Trajadura cultivars from the Galicia region (Spain). Methionol is produced by yeast from methionine, *via* deamination, followed by decarboxylation (Ehrlich reaction); the aldehyde thus formed, 3-(methylthio)-1-propanol (methional), is then reduced to the alcohol (methionol) or oxidised to the acid (3-(methylthio)propionic acid). The reaction of methionol with acetic acid yields 3-(methylthio)propyl acetate (Rauhut, 1993). Baumes, Cordonnier, Nitz, and Drawert (1986) reported

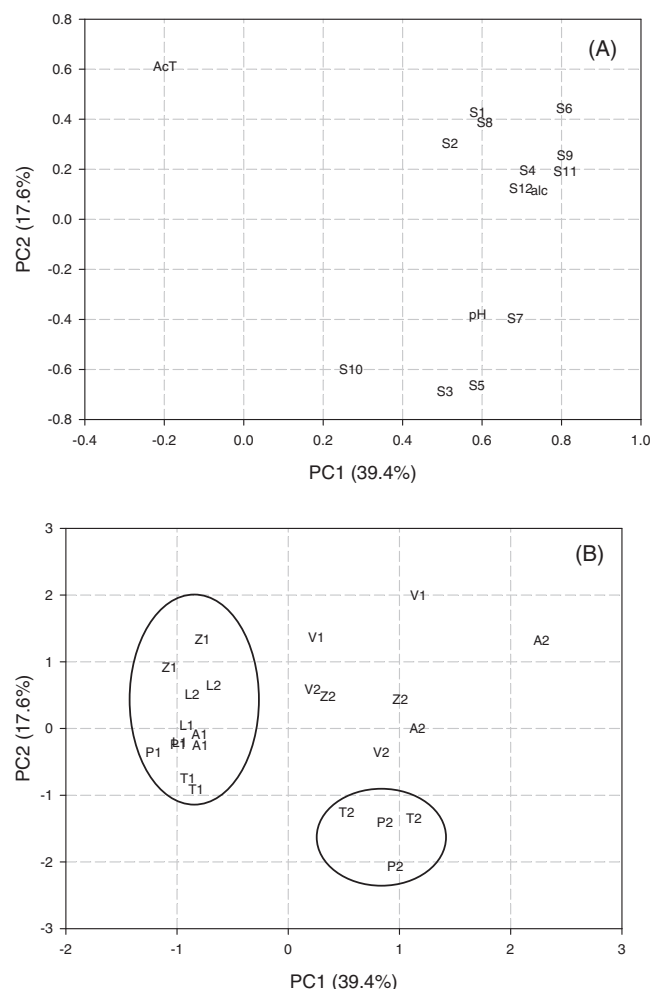


Fig. 1. Principal component analysis: projection of variables and wines on the space defined by the first and second principal components. (A) Variables: S-methyl thioacetate (S1), 2-mercaptoethanol (S2), 2-(methylthio)ethanol (S3), 2-methyltetrahydrothiophen-3-one (S4), 3-(methylthio)propyl acetate (S5), 3-mercapto-1-propanol (S6), methionol (S7), 3-(ethylthio)-1-propanol (S8), 3-methylthiopropionic acid (S9), *N*-3-(methylthiopropyl)acetamide (S10), *cis*- (S11) and *trans*-2-methyltetrahydrothiophen-3-ol (S12), pH, total acidity (AcT) and alcoholic degree (alc). (B) Wines: Trajadura (T), Pedernã (P), Alvarinho (A), Loureiro (L), Azal Branco (Z) and Avesso (V), followed by 1 (1st vintage) or 2 (2nd vintage).

concentrations of methionol from 507 to 998 µg/l in white wines; however, higher values, up to 5000 µg/l, have also been reported in wines (Anocibar Beloqui & Bertrand, 1995; Chatonnet, Dubourdieu, & Boidron, 1992; Fedrizzi, Magno, Badocco, et al., 2007; Fedrizzi, Magno, Moser, Nicolini, & Versini, 2007; Ugliano et al., 2009). The content of methionol increased considerably in wines with reduction defects (Chatonnet et al., 1992; Mestres et al., 2000, 2002), contributing odours of potato, cauliflower and cooked vegetables/cabbage (Table 1).

In general, Loureiro, Trajadura and Pedernã cultivars led to wines with low concentrations of sulphur compounds, being characterised by low contents in *S*-methyl thioacetate, 3-mercapto-1-propanol and 2-mercaptoethanol. *S*-methyl thioacetate concentrations of wines varied between 1.92 and 13.6 µg/l. The highest levels of this compound were obtained in Alvarinho, Avesso and Azal Branco wines. Values of *S*-methyl thioacetate, from 0 to 70 µg/l, have been reported in wines, and concentrations in wines can reach up to 115 µg/l (Fedrizzi, Magno, Badocco, et al., 2007; Mestres et al., 2000). This compound possesses an objectionable odour of rotten/cooked vegetables and sulphurous. In general, the highest contents in 3-mercapto-1-propanol were also obtained for Alvarinho, Azal Branco and Avesso wines (19.9–62.0 µg/l). Loureiro, Trajadura and Pedernã wines showed concentrations of this compound of 9.87–28.4 µg/l. Concentrations lower than 13.5 µg/l were reported in white wines (Lavigne, 1996). 3-Mercapto-1-propanol may have a negative influence on wine aroma at contents higher than its threshold value (Table 1). 2-Mercaptoethanol concentrations varied between 13.1 and 71.5 µg/l; in general, the highest amounts were observed in Azal Branco and Avesso wines. Values varying between 2 and 55 µg/l were reported in white and red wines (Anocibar Beloqui, 1998; Fedrizzi, Magno, Badocco, et al., 2007; Ugliano et al., 2009). Its negative influence on wine quality seems unlikely, because the estimated concentrations found in wines are far below its threshold value.

Loureiro wines were also characterised by significant amounts of dimethyl sulphone, whereas Trajadura wines possessed a high content in 3-(methylthio)propyl acetate and 4-(methylthio)-1-butanol. Significant amounts of *N*-3-(methylthiopropyl)acetamide were also found in Trajadura and Pedernã wines in Year 2. Dimethyl sulphone and *N*-3-(methylthiopropyl)acetamide have no important sensory impact. 3-(Methylthio)propyl acetate presents a mushroom, onion and garlic odour; however, its content in wines never exceeds the threshold value, having little influence on wine quality. Significant amounts of 4-(methylthio)-1-butanol were also observed in Azal Branco wines. Concentrations of this compound in wines varied between 9.85 and 28.7 µg/l. Values up to 22.4 µg/l were also reported in red wines (Ugliano et al., 2009). This compound has chive, onion, garlic, earthy, metallic-bitter or grassy notes, depending on its concentration in wine, but is not considered a negative influence to wine aroma. Its threshold value was found to be higher than its usual content in wines (0–180 µg/l) (Anocibar Beloqui, 1998; Fedrizzi, Magno, Badocco, et al., 2007; Mestres et al., 2002).

The highest values of 3-(ethylthio)-1-propanol were observed in Alvarinho wines in Year 2 (61.0 µg/l) and in Avesso wines in Year 1 (30.8 µg/l). Similar results were reported for other white wines, with concentrations of this compound varying from 6 to 68 µg/l (Baumes et al., 1986; Lavigne, 1996). Significant amounts of 3-methylthiopropionic acid were also found in Alvarinho and Avesso wines. In white wines, 3-methylthiopropionic acid can be detected at concentrations up to 70 µg/l; higher contents, up to 1448 µg/l, were quantified in cloudy wines (Anocibar Beloqui, 1998; Mestres et al., 2000). Karagiannis and Lanaridis (1999) found 3-methylthiopropionic acid concentrations in white wines varying between 13 and 168 µg/l, which were influenced by the yeast strain and the cultivar used. This compound has a perception

threshold of 50 µg/l in hydroalcoholic solution and of 244 µg/l in red Merlot wine, and is characterised by rancid/butter, chocolate and roasted odours (Table 1).

Avesso wines were also characterised by significant amounts of 2-methyltetrahydrothiophen-3-one and *cis*- and *trans*-2-methyltetrahydrothiophen-3-ol. 2-Methyltetrahydrothiophen-3-one contents of wines varied between 42.6 and 245 µg/l. Baumes et al. (1986) detected concentrations of 7–45 µg/l in white wines; however, higher values up to 231 µg/l were reported in Greek white wines and Sauvignon wines, as well as in wines with reduction defects (Chatonnet et al., 1992; Karagiannis & Lanaridis, 1999; Lavigne, Boidron, & Dubourdieu, 1992). This sulphur compound possesses a metallic, butane-like odour and a threshold value of 150 µg/l in white wines. Trajadura and Alvarinho wines also contained significant amounts of *cis*- and *trans*-2-methyltetrahydrothiophen-3-ol in Year 2. *Cis*- and *trans*-2-methyltetrahydrothiophen-3-ol were detected at concentrations of 5–12 µg/l in white wines (Baumes et al., 1986; Rauhut, 1993). Only the *trans*-isomer has been reported as presenting odour activity, similar to onion and chive-garlic.

Besides the high content in 4-(methylthio)-1-butanol, *S*-methyl thioacetate, 3-mercapto-1-propanol and in 2-mercaptoethanol, Azal Branco wines are also characterised by low amounts of 2-(methylthio)ethanol. Low levels of 2-(methylthio)ethanol were also obtained in Loureiro cultivars. Concentrations of this compound lower than 152 µg/l were reported in white wines (Anocibar Beloqui, 1998; Fedrizzi, Magno, Moser, et al., 2007; Karagiannis & Lanaridis, 1999; Lavigne, 1996; Ugliano et al., 2009). These concentrations are lower than the threshold values (Table 1).

A linear discriminant analysis (LDA) was applied to all wines from both vintages in order to optimise their separation according to grape cultivar. All heavy sulphur compounds concentrations were used and contributed to the analysis. Five LDA factors were effective in discriminating the cultivar employed, explaining 100% of the total variance. Fig. 2 shows the projection of the wines in a two-dimensional space. Six groups, representing the wines from each cultivar, are clearly observed.

In conclusions, the concentrations of sulphur compounds found in wines were, in general, below their perception threshold values. Experiments show that wines from the six cultivars could be differentiated according to their sulphur compounds content. Low concentrations of sulphur compounds were found in Loureiro, Trajadura and Pedernã wines. However, Loureiro wines showed significant amounts of dimethyl sulphone, whereas Trajadura

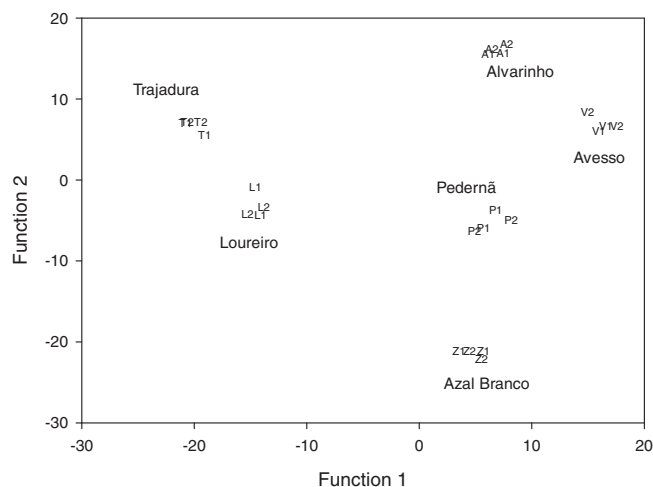


Fig. 2. Linear discriminant analysis: projection of wines from Trajadura (T), Pedernã (P), Alvarinho (A), Loureiro (L), Azal Branco (Z) and Avesso (V), followed by 1 (1st vintage) or 2 (2nd vintage).

wines presented a high content in 3-(methylthio)propyl acetate and 4-(methylthio)-1-butanol. Alvarinho and Avesso wines showed high concentrations of S-methyl thioacetate, 3-mercapto-1-propanol, 3-(ethylthio)-1-propanol and 3-methylthiopropionic acid. Avesso wines also presented significant amounts of 2-methyltetrahydrothiophen-3-one and *cis*- and *trans*-2-methyltetrahydrothiophen-3-ol. Azal Branco wines were characterised by low contents in 3-(methylthio)propyl acetate and 2-(methylthio)ethanol, and high contents in S-methyl thioacetate, 3-mercapto-1-propanol and 2-mercaptoethanol. Some differences were also observed according to the year of vintage and may be due to climatic conditions that affect the nutrient levels of grape musts.

It is known that precursors of sulphur compounds are present in grape musts, and may vary with the variety and origin of grapes. Other factors, like climatic conditions, application of fertilizers, vinification procedures and conservation of wines, must be investigated in order to understand their effect on sulphur compounds synthesis. These parameters are known to influence the amino acid metabolism of yeasts which, in turn, is related to the production of some sulphur compounds.

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