Identification of key-odorants in Sauternes Wines

Identification des composés organoleptiquement actifs des vins de Sauternes

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Abstract : The aim of the present work was to investigate Sauternes wines aromas. The flavor profiles of two wines (vintages 2002 and 2003) were investigated. Key-odorants have been determined by AEDA applied to Amberlite XAD-2 resin extracts. Various complementary techniques were used to identify the compounds (pHMB extraction, chemical synthesis of non-commercial standards, co-injections on two capillary columns, odor description at the sniffing port, GC-MS and GC-PFPD).
Among key-odorants, varietal aromas (α-terpineol, linalool) and fermentation alcohols (3-methylbutanol, β-phenylethanol) and esters (ethyl butyrate, ethyl isovalerate, ethyl hexanoate) are relevant. Maturation in oak barrels provides changes in the aroma profile. Guaiacol, eugenol, vanillin, δ-nonalactone and *cis*-whiskylactone have a FD value ≥ 27 after maturation. Unreduced carbonyles such as *trans*-2-nonenal and β-damascenone can also be issued from oak. Polyfunctional thiols emerge as the most interesting odorants.
Sotolon, previously described as characteristic of noble rot and indicator of wine oxidation, is underestimated in our XAD-2 extract. A specific extraction procedure has been therefore optimized.

Key words: Sauternes wines, aroma, AEDA, sotolon

Introduction

Even if wine aromas are abundantly studied, very few is known about Sauternes wines. These wines are made from Muscadelle, Sauvignon blanc and Semillon grapes contaminated by noble rot. Alcoholic fermentation is limited by adding sulfites (*mutage*) leading to final sugar concentration close to 100 g/L.
Botrytis cinerea has a huge impact on wine constituents. Amongst wine aromas, furfural, benzaldehyde, and phenylacetaldehyde were shown to be metabolites of the fungus (Kikuchi *et al.*, 1983). Terpenic compounds such as linalool can be biotransformed by *Botrytis cinerea* thanks to its hydrolase activity (Aleu and Collado, 2001). The noble rot can also hydrolyse glycosidic terpenes (Ribéreau-Gayon *et al.*, 1998).
Sotolon, found at 5 to 20 ppb level in Sauternes wines, was attributed to noble rot by Masuda *et al.* (1984). However, Sponholz and Huhn (1994) found no correlation between sotolon content and infection rate. According to Cutzach *et al.* (1998), this lactone is an indicator of oxidative wine ageing in sweet fortified wines.
Tominaga (1998) showed that polyfunctional thiols take part in global wine aromas of Sauternes wines. 4-Mercapto-4-methyl-pentane-2-one and 3-mercaptohexanol would be found at concentrations above their threshold value.

Materials and methods

Aroma analysis

The XAD-2 extraction procedure (Lermusieau *et al.*, 2001) was applied on a Sauternes wines (vintages 2002 and 2003). 20 ppm of dodecane were added as internal standard before concentration to 500 μL. The extracts were analysed on gas chromatograph hyphenated with mass spectrometry, FID or olfactometric detection. Complete Aroma Extract Dilution Analysis (AEDA) was performed on XAD-2 wine extracts. A thiol specific procedure using p-HMB (Tominaga, 1998) was also applied. In our case, 250 ml of wine was extracted with ethyl acetate and 12.5 nmol of 4-methoxy-2-methyl-2-mercaptobutane (Oxford Chemicals, UK) was added as internal standard. Gas chromatography coupled with a PFPD detector was used. Compounds were analyzed using a wall-coated open tubular (WCOT) apolar CP-Sil-5CB capillary column

(50 m x 0.32 mm i.d., 1.2 μm film thickness) or a polar FFAP-CB capillary column (25 m x 0.32 μm i.d., 0.3 μm film thickness). The oven temperature was programmed to rise from 36°C to 85°C at 20°C/min, then to 145°C at 1°C/min, and finally to 250°C at 3°C/min.

Recovery factor for XAD-2 extraction

Increasing concentrations of ethyl hexanoate (Fluka, Germany) (from 0.5 to 4 ppm), β-phenylethanol (Fluka, Germany) (from 10 to 80 ppm) and vanillin (Acros, Belgium) (from 0.5 to 4 ppm) were added to wine (vintage 2003) before extraction according to the XAD-2 procedure. The extracts were analysed in GC-MS.

Results and discussion

The analysed wines were a kind gift of Château Guiraud, Sauternes, France. To identify key-odorants in Sauternes wines, vintages 2002 and 2003 were analyzed. Aroma extract dilution analysis (AEDA) was performed on XAD-2 wine extract to determine the impact of volatile compounds. Identifications were realized by co-injections of standards on two capillary columns (CP-Sil5-CB and FFAP-CB), by comparison of mass spectrum data with those of standards or those of the NIST library. pHMB extracts permit to confirm the presence of polyfunctional thiols by GC-olfactometry, GC-PFPD and GC-MS. Table 1 presents the compounds found in at least one wine with a FD value above 27.

Table 1 - Key-odorants in Sauternes wines (Château Guiraud, vintages 2002 and 2003). Aroma Extract Dilution Analysis of XAD-2 wine extract. *: co-elution of 2 compounds. a: detected by GC-PFPD. b: GC-MS in pHMB extract. c: detected by GC-O in pHMB extract.

R.I. CPSil5	R.I. FFAP	Odor	Odorant	FD in 2002 extract on CPSil5	FD in 2003 extract on CPSil5	Identification in the XAD-2 extract by		
						R.I. & Odor on CPSil5	R.I. & Odor on FFAP	GC-MS on CPsil5
(a) Varietal aromas								
1090*	1921	Rose, wine	*beta*-Phenylethanol*	243*	243-729*	X	X	X
	1556		and linalool*			X		X
1179	1706	Floral, musty orange	*alpha*-Terpineol	243	81-243	X	X	
(b) Compounds coming from fermentation								
707	1217	Alcohol, chocolate	3-Methylbutanol	243	729	X	X	X
770	1081	Acid fruit, liquor	Ethyl butyrate	81	81	X	X	X
828	1114	Red fruit	Ethyl isovalerate	243	81	X	X	X
975	1241	Syrup, green apple	Ethyl hexanoate	243	27	X	X	X
1090*	1921	Rose, wine	*beta*-Phenylethanol*	243*	243-729*	X	X	X
	1556		and linalool*			X		X
(c) Influence of oak maturation								
1025	1992	Candy cotton	Furaneol	81	27-81	X	X	
1063	1873	Wood, phenolic, spicy	Guaiacol	81	81-243	X	X	
1133	1497	Cardboard, rubber	*trans*-2-Nonenal	9	243	X	X	X
1281		Sweet, coco, butter	*cis*-Whisky lactone	243	243	X		X
1322	2032	Sweat, coco	*gamma*-Nonalactone	27	27-81	X	X	X
1335	1835	Hay tree, dentist	Eugenol	81	27	X	X	X
1360	2555	Vanilla, cake	Vanillin	81	9-27	X	X	X
1368	1818	Stewed fruit, peach	*beta*-Damascenone	81	81-243	X	X	
(d) Polyfunctional thiols								
804	1112	Hop, spicy	3-Methyl-2-buten-1-thiol (MBT)[c]	81	1	X		
845*	1653	Petroleum, bacon	3-Mercapto-3-methylbutanal (3MMBal)*[,c]	243*	27-81*	X	X	
	1306		and 2-methyl-3-furanthiol (MFT)*[,b,c]			X		
989	1565	Olive, bacon, plastic	3-Mercaptopropyl acetate[a,b,c]	81	27	X	X	
1096	1853	Fruity, rhubarb	3-Mercaptohexanol[a,b,c]	81	729	X	X	
1118	1659	Fruity, lemon	3-Mercaptoheptanal[c]	2187	81	X		
(e) Other compounds								
1036	1668	Fruity	Acetophenone	2187	nd	X		X

R.I. CPSil5	R.I. FFAP	Odor	Odorant	FD in 2002 extract on CPSil5	FD in 2003 extract on CPSil5	Identification in the XAD-2 extract by R.I. & Odor on CPSil5	R.I. & Odor on FFAP	GC-MS on CPsil5
1068	2213	Caramel, praline, curry	Sotolon	243	81-243	X	X	
(f) Unidentified compounds								
730		Crystallized fruit	Unknown	729	729			
902		Plastic	Unknown	81	nd			
1104		Candy cotton, cake	Unknown	2187	81			
1138	1667	Unpleasant, rubber	Unknown	81	27-81			
1147	1649	Fruity	Unknown	81	1			
1217	1985	Unpleasant, floral	Unknown	81	9-27			
1270		Spicy, floral	Unknown	243	27			
1305		Honey, acid drops	Unknown	243	243			
1363		Synthetic material	Unknown	27	81			
1396		Soap, floral	Unknown	27	81			
1417		Cleaning materials	Unknown	81	nd			
1419		Sweet, Sauternes wine	Unknown	27	81			
1430	1930	Sweet, marmalade	Unknown	27	81			

Amongst varietal aromas, *alpha*-terpineol (floral, musty orange with FD = 81-243) and linalool participate in the global aroma profile. However, linalool co-elutes with β-phenylethanol; its impact must be still investigated.

Yeast metabolism logically influences Sauternes wines aromas. Fermentation alcohols as 3-methylbutanol (alcohol, chocolate with FD = 243-729) and β-phenylethanol (co-eluting with linalool; roses, wine with FD = 243-729), and fermentation esters (ethyl butyrate with FD = 81; ethyl isovalerate with FD = 81-243; ethyl hexanoate with FD = 27-243) provide fruity aromas to wine. Additional experiments on musts confirmed that 3-methylbutanol, ethyl butyrate and ethyl hexanoate are issued from yeast fermentation.

After fermentation in oak barrels, our Sauternes wine samples maturated for two years. This step in the vinification process provides wood-extractable compounds and allows oxidative ageing. Guaiacol (FD = 81-243), eugenol (FD = 27-81) and vanillin (FD = 9-81), coming from thermic degradation of wood lignin, provide spicy, hay-tree and vanilla-flavoured aromas to wine. The presence of furaneol (candy cotton with FD = 27-81), a Maillard reaction product, is worth stressing. Wood-extractable lactones as δ-nonalactone (FD = 27-81) and *cis*-whisky lactone (FD = 243) with their coco-like aroma, are relevant. Surprisingly, unreduced carbonyles such as *trans*-2-nonenal (cardboard with FD = 9-243) and β-damascenone (stewed fruit with FD = 81-243) are amongst the most significant aroma. *trans*-2-Nonenal is known to be issued from fatty acid oxidation (Noël *et al.*, 1999); it can be brought by fresh wood (Ribéreau-Gayon, 1998). β-damascenone is most probably derived from wood carotenoids (Jarauta *et al.*, 2005).

A large series of polyfunctional thiols with their fruity, spicy aromas have been found in the Sauternes extracts. 3-Mercaptohexanol (rhubarb with FD = 81-729) was already identified in Sauternes wines by Tominaga (1998). 3-Mercaptopropyl acetate (bacon with FD = 27-81) was found in Semillon and Sauvignon grapes (Lavigne *et al.*, 1998) while 2-methyl-3-furanthiol (bacon) was previously identified in red wines (Bouchilloux *et al.*, 1998). 3-Methyl-2-buten-1-thiol (feed, spicy with FD = 1-81) and 3-mercapto-3-methylbutanal (bacon) are most probably synthesized during fermentation from the same precursor, 3-methyl-2-butenal, which reacts with hydrogen sulfide excreted by yeast (Vermeulen and Collin, 2002; Vermeulen, 2005). The presence of another mercaptoaldehyde, 3-mercaptoheptanal (fruity, lemon), is to underline.

Some unidentified compounds with high FD values provide pleasant aromas to the extracts : R.I. $_{CPSil5}$ = 730 (crystallized fruit, acid drops), R.I. $_{CPSil5}$ = 1104 (candy cotton, cake), R.I. $_{CPSil5}$ = 1305 (honey, acid drops).

The recovery factors of ethyl hexanoate, β-phenylethanol and vanillin were calculated for our XAD-2 extraction procedure. The high efficiency of the extraction (83%, 81%, 100%, respectively) explains the high representativity of the analysed extracts.

Only sotolon revealed much less extracted by the XAD-2 procedure. Logically, this small lactone is not well retained on the apolar resin; its impact could be therefore underestimated (caramel, curry with FD = 81-243).

A specific extraction of sotolon was optimized: the aqueous eluate from our XAD-2 resin was extracted 2 times with 20 ml diethyl ether and analyzed by GC-MS-SIM (selected ions: 83 and 128) after concentration to 1,5 mL. In a wine model medium (hydroalcoholic solution 13:83 (v/v), glucose 30 g/L, fructose 70 g/L, glycerol 12 g/L, tartaric acid (3 g/L), citric acid (0,3 g/L), pH 4,1), the recovery factor revealed significantly increased by this way (80%). Applied to Sauternes wines, the concentration of sotolon reached 100 ppb.

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