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Role of Certain Volatile Thiols in the Bouquet of Aged Champagne Wines

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A method for the specific extraction of volatile thiols by use of *p*-hydroxymercuribenzoate has made it possible to identify certain flavor-active volatile thiols in Champagne wines. Benzenemethanethiol, 2-furanmethanethiol, and ethyl 3-mercaptopropionate were present in these wines at concentrations considerably higher than their perception thresholds. Their concentrations increased gradually in proportion to the bottle aging period and sharply as a result of disgorging. The contribution of these volatile thiols to the empyreumatic nuances of the bouquet of aged Champagne wines was demonstrated for the first time.

KEYWORDS: 2-Furamethanethiol; benzenemethanethiol; ethyl 3-mercaptopropionate; Champagne wine; aging; bouquet

INTRODUCTION

The aroma of some old Champagne wines prized by connoisseurs differs from that of younger wines by a marked empyreumatic character, reminiscent of roasted coffee beans and toast, brioche, or toasted crusts (*1*−*2*). The volatile compounds (4,5-dimethyltetrahydrofuran-2,3-dione, 5-ethoxybutyrolactone, benzaldehyde, and diacetyl) previously identified in old Champagne wines (*3*−*4*) did not account for the empyreumatic nuances in their bouquet.

We recently demonstrated the contribution of furanmethanethiol to the toasty aroma of some red and white wines aged in oak barrels (*5*). This compound has also been reported in some Champagnes (*6*). However, the development of furanmethanethiol in Champagne during aging and the possible contribution of other empyreumatic volatile thiols to its bouquet had not previously been investigated.

This article reports on the identification of certain volatile thiols in Champagne and the development of these compounds during bottle aging. The impact of disgorging on concentrations was also studied.

MATERIALS AND METHODS

Chemicals. 2-Furamethanethiol, 2-methyl-3-furanthiol, ethyl 3-mercaptopropionate, and benzenemethanethiol were supplied by Sigma−Aldrich. Ethyl 2-mercaptopropionate was obtained from Interchim (03103 Montluçon, France). 4-Methoxy-2-methyl-2-mercaptobutane used as an internal standard for quantitation was provided by Oxford Chemicals.

Wines Analyzed. The Champagne wines analyzed (*CRISTAL* and *GRAND SIECLE*) were kindly donated by the Louis Roederer and Laurent Perrier Champagne companies. The wines consist of Chardonnay and Pinot noir (≅1:1). Details of the samples (vintage, disgorging date, etc.) are shown in **Table 1**.

Identification and Assays of the Volatile Thiols. The volatile thiols were purified and assayed according to the method described by Tominaga et al. (*5*), with ethyl acetate instead of dichloromethane used to extract total volatile compounds from wine as follows. A volume of 500 mL of wine containing 2.5 nmol of 4-methoxy-2-methyl-2-mercaptobutane as an internal standard was extracted with 2 successive additions of 100 mL of ethyl acetate in a 1 L flask with magnetic stirring for 5 min each time. The combined organic phases were centrifuged for 5 min at 3800*g* to break the emulsion and separated in a separating funnel. The organic phase obtained was then extracted with two successive additions of 20 mL of a *p*-hydroxymercuribenzoate solution (1 mM in Tris at 0.2 M) for 5 min each time. The two aqueous phases, from the extraction, were combined and then loaded into a strongly basic anion-exchanger column (1.5 × 3 cm) (Dowex1, Sigma, 1X2−100). The column was then washed with 50 mL of sodium acetate buffer (0.05 M, pH 6). The volatile thiols were released from the complex thiol-*p*-hydroxymercuribenzoate fixed on the column by percolating with a cysteine solution (640 mg/60 mL) adjusted to pH 7, previously purified of any volatile contaminants by repeated extractions with dichloromethane (5 mL × 3). The eluate containing the volatile thiols released from the column by cysteine was collected in a 100 mL flask and extracted with 4- and 3-mL portions of dichloromethane for 5 min each, with magnetic stirring. The organic phases were collected, dried on anhydrous sodium sulfate, and then concentrated under nitrogen flow in a 10 mL graduated tube to approximately 500 μL. The concentrate was then transferred to a 1 mL vial and concentrated to 25 μL.

The volatile thiols were identified by comparing the retention time and the spectrum obtained in SCAN mode with those of reference compounds. They were quantified by GC/MS in SIM mode (see below).

Aldehyde and Alcohol Assays. Furfural, benzaldehyde, and furfuryl alcohol were assayed in the wines according to the method described by Boidron et al. (*7*).

GC/Olfactometry, FPD, and MS. The chromatography conditions were the same as those described by Tominaga et al. (*8*), replacing the BP-20 column with BPX-35 (SGE, 50 m × 0.22 mm, 0.25 μm).

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Table 1. List of Champagne Wines Analyzed[a]

samples	vintage	drafting date	disgorging date	time before disgorging (years)	time after disgorging (years)	total bottle aging time (years)
			Louis Roederer			
LR-1	2000			0	0	0
LR-2	1995	1996	2001	5	0.5	5.5
LR-2ND	1995	1996	b	5.5	0	5.5
LR-3	1993	1994	1998	4	3	7
LR-3ND	1993	1994	b	7	0	7
LR-4	1990	1991	1998	7	3	10
LR-4ND	1990	1991	b	10	0	10
LR-5	1989	1990	1997	7	4	11
LR-5ND	1989	1990	b	11	0	11
LR-6	1988	1989	1997	8	4	12
LR-7	1985	1986	1989	3	12	15
LR-8	1983	1984	1989	5	12	17
LR-9	1982	1983	1988	5	13	18
LR-10	1981	1982	1986	4	15	19
LR-11	1979	1980	1984	4	17	21
			Laurent Perrier			
LP-1	1996	1997	2000	3	1	4
LP-2	1995	1996	2000	4	1	5
LP-3	1988	1989	2000	11	1	12
LP-4	1985	1986	2000	14	1	15
LP-5	1982	1983	2000	17	1	18
LP-6	1979	1980	2000	20	1	21
LP-7	1976	1977	2000	23	1	24
LP-8	1973	1974	2000	26	1	27

[a] Analyzed in June 2001. [b] Nondisgorged.

Quantitation was carried out in SIM mode, selecting the ions as follows: $m/z = 53$, 81, and 114 for 2-furanmethanethiol, $m/z = 61$, 88, and 134 for ethyl 3-mercaptopropionate, and $m/z = 65$, 91, and 124 for benzenemethanethiol. The aldehydes and alcohols were assayed on a BP-20 column (SGE, 50 m × 0.22 mm, 0.25 μm) in SIM mode, selecting the ions as follows: $m/z = 95$ for furfural, $m/z = 106$ for benzaldehyde, and $m/z = 81$ for furfuryl alcohol.

RESULTS AND DISCUSSION

Certain Volatile Thiols in Champagne Identified by GC/MS. The volatile thiols in Champagne wines were specifically extracted and purified by the method mentioned above and analyzed by GC/olfactometry and GC/FPD. The GC/FPD chromatogram (**Figure 1**) of an old Champagne wine (Laurent Perrier, 1973 vintage) had a large peak (peak 2), corresponding in olfactometry to a highly odoriferous zone that smells of roasted coffee beans. Peaks 1 and 5 also had odors remarkably evocative of cooked and smoked meat, respectively. The corresponding compounds were identified by comparing the retention times and spectra obtained by electron impact with those of reference compounds. These were 2-methyl-3-furanthiol (peak 1), 2-furanmethanethiol (peak 2), and benzenemethanethiol (peak 5). The following compounds, hardly perceptible by olfactometry, if at all, were also identified: ethyl 2-mercaptopropionate (peak 3), ethyl 3-mercaptopropionate (peak 4), and 3-mercaptohexan-1-ol (peak 6).

It is well-known that 2-furanmethanethiol and 2-methyl-3-furanthiol are formed in certain foods during cooking, via a Maillard reaction (*9−12*). Some still red and white wines and sparkling white wines made from *Vitis vinifera* grapes have also been found to contain 2-furanmethanethiol (*5*, *6*). 2-Methyl-3-furanthiol has only been reported in a few red Bordeaux and Rioja wines (*13−15*). However, the existence of this thiol and its dimer, bis(2-methyl-3-furyl) disulfide, in Champagne wines, was suspected by Etievant (*16*). Benzenemethanethiol, with its empyreumatic odor, was recently identified in several wines, particularly Chardonnay (*17*). 3-Mercaptohexan-1-ol, initially identified in passion fruit (*18*) is also present in several red and white *Vitis vinifera* wines (*19−22*). It has long been known that apple juice and strawberries contain ethyl 2-mercaptopropionate (*23*, *24*) and that ethyl 3-mercaptopropionate is found in *Vitis labrusca* wines (*25*). While 2-furanmethanethiol had already been reported (*6*), the other five volatile thiols were identified in Champagne wines for the first time. However, although 2-mercaptothiophene has recently been reported in Champagne wines (*6*), it was not identified under our analytical conditions.

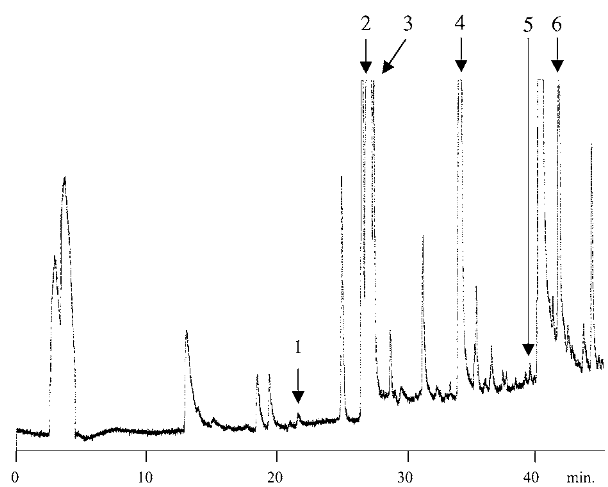

Figure 1. Chromatogram (GC/FPD) of volatile thiols extracted from an old champagne wine (Laurent Perrier, 1973 vintage). Peak 1, 2-methyl-3-furanthiol; peak 2, 2-furanmethanethiol; peak 3, ethyl 2-mercaptopropionate; peak 4, ethyl 3-mercaptopropionate; peak 5, benzenemethanethiol; peak 6, 3-mercaptohexan-1-ol.

Assaying the Volatile Thiols Identified in Champagne Wines. The five volatile thiols identified in Champagne wines were assayed. It was not possible to assay 2-methyl-3-furanthiol accurately, due to little recovery under our extraction conditions with *p*-hydroxymercuribenzoate. **Table 2** shows the concentra-

Table 2. Perception Thresholds (ng/L) of the Volatile Thiols Identified and Their Concentrations (ng/L) in Champagne Wines

		concentration (ng/L)	
compound	perception threshold (ng/L)	Louis Roederer wines	Laurent Perrier wines
2-furanmethanethiol	0.4[a]	2–360	300–5500
ethyl 2-mercaptopropionate	500[b]	50–200	100–800
ethyl 3-mercaptopropionate	200[c]	40–5200	2400–12 000
benzenemethanethiol	0.3[d]	10–100	30–400
3-mercaptohexan-1-ol	60[e]	250–640	250–510

[a] Reference 5. [b] Reference 29. [c] Reference 25. [d] Reference 17. [e] Reference 19.

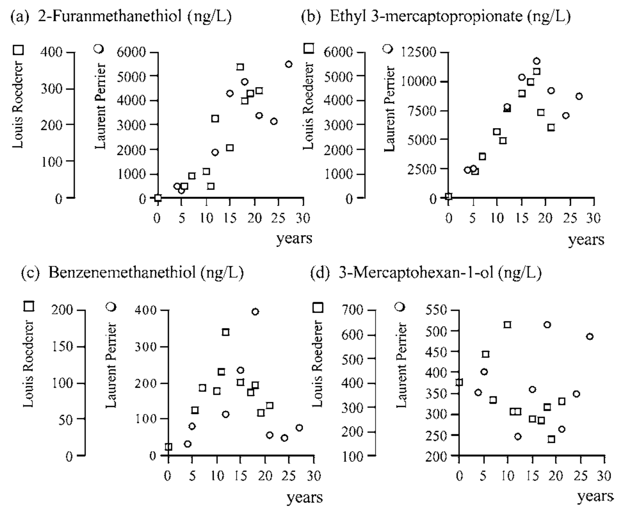

Figure 2. Development of the volatile thiols content during bottle aging in champagne wines produced by Louis Roederer (□) and Laurent Perrier (○).

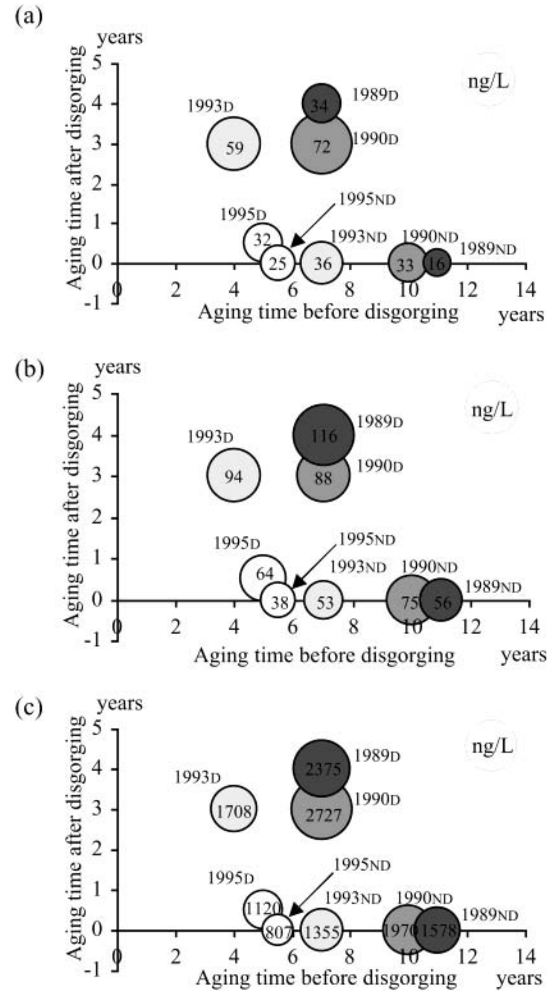

Figure 3. Influence of disgorging on the concentrations of three volatile thiols in champagne wines: (a) 2-furanmethanethiol; (b) benzenemethanethiol; (c) ethyl 3-mercaptopropionate.

tion intervals of the five volatile thiols obtained from 11 Louis Roederer wines (LR1−11) and eight Laurent Perrier wines (LP1−8).

The 2-furanmethanethiol and benzenemethanethiol content of all the wines analyzed, as well as the ethyl 3-mercaptopropionate content of some Louis Roederer wines and all the Laurent Perrier wines, were considerably higher than their perception thresholds (**Table 2**). These three compounds have not been found at such high concentrations in other red and still white wines analyzed to date. These compounds, therefore, have an undeniable impact on the aroma of these Champagne wines. The 3-mercaptohexan-1-ol content in all the wines analyzed was 4−10 times the perception threshold. It may well contribute to the fruity aroma of Champagne wine. The presence of 3-mercaptohexan-1-ol has also been reported in wines made from a number of grape varieties (*19*−*22*). However, the ethyl 2-mercaptopropionate content of most of the wines analyzed was significantly lower than the perception threshold, so it is unlikely to play a role in wine aroma.

Impact of Bottle Aging Time on Concentrations of Volatile Thiols. The development of flavor-active volatile thiols according to the total bottle-aging period (before and after disgorging) was studied by analyzing different vintages of the same type of Champagne at the same time. The 2-furanmethanethiol content in the wines from both companies increased in proportion to bottle aging time (**Figure 2a**). However, the ethyl 3-mercaptopropionate and benzenemethanethiol content of the wines peaked after 13−15 years and then decreased (**Figure 2b,c**). There was no significant correlation between the 3-mercaptohexan-1-ol content and bottle aging time (**Figure 2d**). Apparently, ethyl 3-mercaptopropionate, benzenemethanethiol, and 2-furanmethanethiol make a real contribution to the empyreumatic character of the bouquet of old Champagne wine. The observation that old Champagne wines (*1*) have more pronounced toasty aromas may be explained by the presence of these three volatile thiols.

Impact of Disgorging on the Volatile Thiol Contents. Disgorging to remove the yeast deposit from the wine is a vital stage in the bottle aging of Champagne. The disgorging date, which determines the length of time the Champagne is aged on its lees, varies according to the producer, the type of Champagne, and commercial considerations. The impact of disgorging on the 2-furanmethanethiol, benzenemethanethiol, and ethyl 3-mercaptopropionate concentrations was therefore investigated as follows. Four pairs of Louis Roederer Champagne wines were compared. One sample in each pair had been disgorged at an earlier date, while the other had never been disgorged. On the date the analyses were carried out, the four Champagne wines had been disgorged 6 months, 3 years, and 4 years earlier. The disgorged samples were labeled LR2−5 and the corresponding nondisgorged Champagnes LR2ND−5ND (**Table 1**). **Figure 3** shows the volatile thiol concentrations (○) in the nondisgorged and disgorged Champagne wines. Nondisgorged samples (ND) are located on the *x*-axis. Disgorged samples (D) are distributed above all nondisgorged samples according to aging time after disgorging. The concentrations of 2-furanmethanethiol, ben-

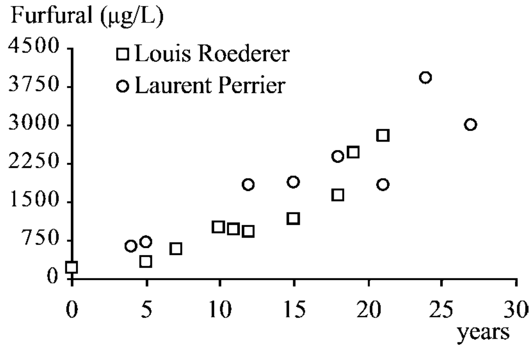

Figure 4. Evolution of furfural content during bottle aging in champagne wines produced by Louis Roederer (□) and Laurent Perrier (○).

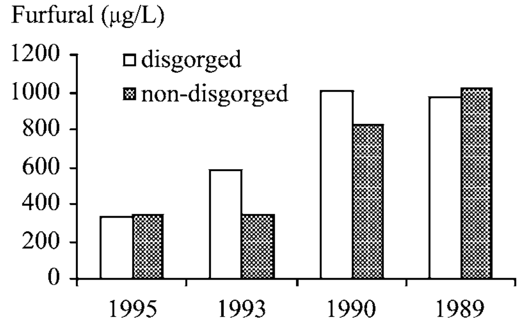

Figure 5. Comparison of the furfural contents of disgorged and nondisgorged champagne wines.

zenemethanethiol, and ethyl 3-mercaptopropionate in the disgorged samples were significantly higher than those of the corresponding nondisgorged samples (**Figure 3**). Apparently, these volatile thiols develop more rapidly after disgorging. In fact, the concentrations of empyreumatic volatile thiols correlated with both the total bottle aging time and the aging time after disgorging. There was no correlation between the volatile thiol contents in Louis Roederer Champagnes and aging time prior to disgorging (results not shown). However, the volatile thiol content of the Laurent Perrier samples, all disgorged 1 year before analysis, increased in proportion to the aging time prior to disgorging.

Evolution of Furfural during Bottle Aging. It is well-known that furfural plays a major role in 2-furanmethanethiol formation in roasting coffee (*9*, *26*). In fact, this aldehyde is precursor of 2-furanmethanethiol formed in barrel-fermented white wines (*27*, *28*). In the Champagne wines we analyzed, the furfural content increased in proportion to the bottle aging time (**Figure 4**). It is, therefore, likely that this compound contributes to the formation of 2-furanmethanethiol. However, the fact that the Louis Roederer and Laurent Perrier Champagnes had very different 2-furanmethanethiol contents, whereas their furfural contents were comparable, suggests that one or more other parameters are involved in 2-furanmethanethiol formation and/or stability. This hypothesis is backed up by the fact that disgorged Champagne wines did not necessarily contain more furfural than nondisgorged samples (**Figure 5**), whereas they had a higher 2-furanmethanethiol content (**Figure 3**). Furfuryl alcohol contents in all wines analyzed were very low (0−50 μg/L). The increase in benzaldehyde content during bottle aging reported by Loyaux et al. (*4*) was not confirmed in our samples (data not shown).

CONCLUSION

Champagne wines contain certain flavor active volatile thiols (2-furanmethanethiol, benzenemethanethiol, and ethyl 3-mercaptopropionate) at concentrations considerably higher than their perception thresholds. These volatile thiols are expected to contribute to the empyreumatic nuance in the bouquet of old Champagne wines. It was also demonstrated that disgorging leads to a significant increase in the concentrations of these compounds in Champagne. The formation mechanisms of these flavor active compounds have not yet been elucidated.

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