# OLFACTORY IMPACT OF DIMETHYL SULFIDE ON RED WINE FRUITY ESTERS AROMA EXPRESSION IN MODEL SOLUTION

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## Abstract

**Aim**: The goal of this study was to evaluate the impact of dimethyl sulfide (DMS) on the fruity aroma of a complex mixture containing 12 red wine esters.

**Methods and results**: Aromatic reconstitutions were prepared using pure commercial esters and acetates at the average concentrations found in red wines, as well as several concentrations of DMS. The olfactory thresholds of DMS in such matrices were: 1.74 µg/L in dilute alcohol solution, 2.80 µg/L in fruity aromatic reconstitution (12 esters) in dilute alcohol solution, and 3.67 µg/L in fruity aromatic reconstitution in dearomatized red wine. The « olfactory threshold » of the fruity pool, consisting of the 12 esters, was calculated in two different matrices: dilute alcohol solution and dilute alcohol solution supplemented with 5 µg/L DMS. The presence of DMS in the mixture led to significantly decreased of the « olfactory threshold » of the fruity pool, highlighting its effect in increasing overall aroma intensity. Sensory profiles were then evaluated to investigate the qualitative impact of DMS on fruity aroma perception.

**Conclusions**: These results confirmed the sensory importance of DMS, suggesting that it was an active contributor to the black-berry fruit nuances in the fruity matrix studied. This compound participated, both quantitatively and qualitatively, in modulating black-berry fruit aroma and, more specifically, in enhancing blackcurrant aroma.

**Significance and impact of the study**: Studies investigating fruity aromas in red wines over the past decade have discussed the implication of DMS in fruity aroma expression. Although DMS does not present fruity aromas, this study clearly shows its indirect impact on fruity aroma expression, via particular perceptive interactions, in fruity model mixture.

**Key words**: dimethyl sulfide, esters, fruity aroma, perceptive interactions, aroma enhancer, blackcurrant

## Résumé

**Objectif**: Le but de cette étude était d'évaluer l'impact du sulfure de diméthyle (DMS) sur l'arôme fruité d'un mélange complexe contenant 12 esters.

**Méthodes et résultats**: Des reconstitutions aromatiques ont été réalisées à l'aide d'esters éthyliques et d'acétates commerciaux aux concentrations moyennes rencontrées dans les vins rouges, ainsi que du DMS à différentes concentrations. Le seuil de perception du DMS a été déterminé dans différentes matrices: 1,74 µg/L en solution hydroalcoolique ; 2,80 µg/L dans la reconstitution aromatique fruitée (12 esters) préparée en solution hydroalcoolique ; et 3,67 µg/L dans la reconstitution aromatique fruitée préparée dans un vin rouge désaromatisé. Le « seuil de perception » du pool fruité constitué des 12 esters a été mesuré dans deux matrices différentes: une solution hydroalcoolique et une solution hydroalcoolique supplémentée avec 5 µg/L de DMS. La présence du DMS dans le mélange diminue significativement le « seuil de perception » du pool fruité. Des profils sensoriels ont été effectués afin d'évaluer l'impact qualitatif du DMS sur la perception de l'arôme fruité.

**Conclusions**: Ces résultats confirment l'impact sensoriel du DMS, démontrant son implication dans la perception des notes de cassis après ajout au pool fruité étudié. Ce composé participe quantitativement et qualitativement à une augmentation de la perception des notes fruits noirs et plus spécialement des notes cassis.

**Importance et impact de l'étude**: L'impact du DMS sur l'arôme fruité des vins rouges a déjà été discuté dans la littérature. Cette étude apporte des éléments nouveaux puisqu'elle démontre de manière précise, en milieu modèle-fruité, l'implication de ce composé dans des interactions sensorielles conduisant à la perception d'une note cassis. Le DMS ne présentant pas de nuances fruitées, cette étude confirme son impact indirect sur l'expression de l'arôme fruité.

**Mots clés**: sulfure de diméthyle, esters, arôme fruité, interactions perceptives, exhausteur, cassis

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## INTRODUCTION

Previous research on the fruity character of red wines highlighted the role of ethyl esters and acetates (Etiévant, 1991; Bertrand *et al.*, 1995; Pineau *et al.*, 2009; Falcao *et al.*, 2012; Lytra *et al.*, 2012a; Lytra *et al.*, 2012b), produced by yeast metabolism during alcoholic fermentation, a phase where red- and black-berry fruit aromas are formed (Pineau *et al.*, 2011). The literature provides evidence that, besides these esters, other compounds that do not necessarily present fruity aromas may have an important impact on the overall fruity aroma of wine. For example, furaneol and homofuraneol, with strong caramel aromas, seem to have an enhancing effect on the perception of red-berry fruit aroma (Kotseridis and Baumes, 2000). In addition, some thiols (3-sulfanylhexan-1-ol) (Bouchilloux *et al.*, 1998) and other volatile sulfur compounds (dimethyl sulfide) may also affect the perception of fruity aroma in red wines (Anocibar-Beloqui *et al.*, 1996; Segurel *et al.*, 2004; Escudero *et al.*, 2007). Some C13-norisoprenoids, such as β-damascenone, are also generally considered to affect red wine fruity aroma (Escudero *et al.*, 2007; Pineau *et al.*, 2007). Finally, in complex mixture, the direct role of diacetyl in fruity expression has recently been highlighted, as well as the indirect roles of acetoin, acetic acid, and γ-butyrolactone (Lytra *et al.*, 2012b).

Dimethyl sulfide (DMS), a light sulfur compound, has been described in a wide range of food products: cooked meat (Jensen *et al.*, 2002) and cheese (Carbonell *et al.*, 2002) as well as tomatoes (Buttery *et al.*, 1971), citrus (Shaw *et al.*, 1980), melons (Homatidou *et al.*, 1992), truffles (Diaz *et al.*, 2003), cereals (Ren, 2001), and some vegetables (Ulrich *et al.*, 2001; Tulio *et al.*, 2002). Its presence in beer and wine has also been reported (Loubser and Du Plessis, 1976).

The olfactory threshold of DMS was established at 27 µg/L in red wine (Anocibar-Beloqui *et al.*, 1996) but seems to be strongly dependant on the matrix and tasting panel, as it has also been estimated at 30 to 45 µg/L (Meilgaard, 1982) and 22 to 60 µg/L (Etiévant, 1991). At the end of alcoholic fermentation, it is present at relatively low levels - generally well below its olfactory threshold - but may reach high concentrations in certain wines during bottle aging, up to mg/L levels (Marais, 1979; de Mora *et al.*, 1993; Anocibar-Beloqui, 1998; Loscos *et al.*, 2008). For example, the study of 17 commercial wines, aged from 1 to 21 years old, showed DMS levels ranging from 3 µg/L in younger wines to 711 µg/L in older vintages (Dagan, 2006).

Anocibar-Beloqui *et al.* (1996) reported that the addition of DMS at concentrations above its olfactory threshold conferred blackcurrant and raspberry notes to some young wines. These observations were confirmed in Grenache and Syrah wines by Segurel *et al.* (2004). Escudero *et al.* (2007) suggested that DMS played the role of fruity flavor enhancer by observing that, while 10 µg/L of this compound was not directly perceived in dearomatized wine, it conferred «sweet-fruity» or «green olive» notes to more complex mixtures.

In older wines, especially those aged in oak barrels, the addition of DMS may produce truffle or hay nuances, and at excessive concentrations, result in an unpleasant green olive odor (Anocibar-Beloqui *et al.*, 1996). The results of Segurel *et al.* (2004) concerning Grenache and Syrah wines confirmed the appearance of similar «black olive» and «truffle» notes. The impact of DMS during aging may also be assessed positively, as in the «reduction» bouquet of great red and late harvest white wines (Spedding and Raut, 1982; Anocibar-Beloqui, 1998). It is, however, perceived rather negatively in young white wines (Goniak and Noble, 1987).

The main goal of this work was to evaluate the impact of DMS on wine fruity aroma expression. Various aromatic reconstitutions involving DMS, together with the 12-ester fruity pool, previously described by Pineau *et al.* (2009) and Lytra *et al.* (2013), were subjected to sensory analysis to determine the quantitative and qualitative contribution of DMS.

## MATERIALS AND METHODS

### 1. Chemicals and odorant stimuli

Absolute ethanol (analytical grade, 99.97%, Scharlau Chemie S.A, Barcelona, Spain) was distilled before use. Sodium sulfate (99%) was provided by Scharlau Chemie S.A (Barcelona, Spain). Microfiltered water was obtained using a Milli-Q Plus water system (resistivity: 18.2 MΩ cm, Millipore, Saint-Quentin-en-Yvelines, France). Standard grade compounds were obtained from commercial sources, as follows: ethyl propanoate, ethyl 2-methylpropanoate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl hexanoate, ethyl octanoate, ethyl 3-hydroxybutanoate, 2-methylpropyl acetate, butyl acetate, hexyl acetate, DMS, and thiophene from Sigma–Aldrich (Saint-Quentin-Fallavier, France); 3-methylbutyl acetate from VWR-Prolabo (Fontenay-sous-Bois, France); and D-ethyl 2-hydroxy-4-methylpentanoate and L- ethyl 2-hydroxy-4-methylpentanoate from Hangzhou Imaginechem Co., Ltd (Hangzhou, China).

## 2. Aromatic reconstitution

For aromatic reconstitutions (AR), the various compounds were blended together at the average concentrations found in red wines (**Table 1**) (Pineau *et al.*, 2009; Antalick *et al.*, 2010), in double-distilled ethanol and microfiltered water, to obtain an ethanol level of 12% (v/v) (pH adjusted to 3.5 with tartaric acid) (Lytra *et al.*, 2013). In addition, the tested levels of DMS were representative of those found in oenological conditions (Dagan, 2006). Dearomatized red wine (DRW) was prepared according to the method described by Lytra *et al.* (2012a) by evaporating red wine to two-thirds of original volume using a Rotavapor (Laborota 4010 digital Rotary Evaporator, Heidolph, Germany) with a 20 °C bath temperature. The liquid was then mixed with double-distilled ethanol and microfiltered water to reproduce the alcohol concentration and volume of the original wine (pH adjusted to 3.5 with tartaric acid). Then DRW (1000 mL) was supplemented with 5 g LiChrolut EN resin (40−120 µm) and stirred for 12 h. The solution was filtered and the same protocol was repeated in order to eliminate all traces of DMS and esters. The resulting DRW had a very low-intensity neutral aroma.

## 3. Gas chromatography-olfactometry (GC-O) analysis of reference compounds

GC-O analyses were carried out to ensure that the high-purity reference compounds did not contain any odoriferous impurities and to ascertain that the compound considered was responsible for the odor properties identified. Olfactometry analyses were carried out using an HP-6890 gas chromatograph (Hewlett–Packard, Wilmington, DE, USA) equipped with a flame ionization detector (FID) and a sniffing port (ODO-I SGE, Ringwood, Australia), connected by a flow-splitter to the column exit. GC effluent was combined with humidified $N_2$ (Air Liquide, France) at the bottom of the glass-sniffing nose (SGE, Victoria, Australia) to avoid nasal dehydration. Samples containing less than 0.2 µL of each pure odorant were injected in splitless-split mode (injector temperature: 240 °C, splitless time: 30 s, split flow: 50 mL/min), using a BP20 column (SGE, Ringwood, Australia, 50 m x 0.22 mm i.d., film thickness: 0.25 µm). The oven was programmed at 40 °C for the first minute, then heated at a rate of 10 °C/min up to a final isotherm at 220 °C for 10 minutes (total sniffing time: 29 min). The carrier gas was hydrogen 5.5 (Air Liquide, France) with a column head pressure of 15 psi.

## 4. DMS analysis

Chromatographic conditions and sample preparation were as inspired by Anocibar-Beloqui (1998). A 100-mL sample was placed in a 125-mL vial at room temperature and 10 µL of the internal standard (thiophene 330 mg/L) were added. The vial was then sealed using a screw-top cap with a Teflon-faced septum. After 24 hours at 22 °C and away from direct light, 1 mL of gaseous phase was injected using the headspace technique (HS). DMS was assayed by injection into an HP-5890 gas chromatograph coupled to a flame photometric detector (injector temperature: 70 °C, interface temperature: 150 °C), using an HP5 column (Crosslinked 5% Ph ME silicone, 30 m × 0.53 mm i.d., film thickness: 5.0 µm). The oven was programmed at 30 °C for the first minute, raised to 100 °C at 10 °C/min, and, finally, increased at 20 °C/min to a final isotherm at 180 °C. The carrier gas was hydrogen 5.5 (Air Liquide, France).

## 5. Sensory analyses

*General conditions.* Sensory analyses were performed as described by Martin and de Revel (1999). Samples were evaluated at controlled room temperature (20 °C), in individual booths, using covered, black ISO glasses (ISO 3591, 1977) containing about 50 mL of liquid and coded with three-digit random numbers. Sessions lasted approximately 5 minutes.

*Sensory panel.* Panel 1consisted of 16 judges, 7 males and 9 females, aged 25.6 ± 7.4 (mean ± SD). Panel 2 consisted of 8 judges, 2 males and 6 females, aged 27.3 ± 5.2 (mean ± SD). All panelists were research laboratory staff at ISVV, Bordeaux University, selected for their experience in assessing fruity aromas in red wines. Berry-fruit syrups and liqueurs (blueberry, blackberry, blackcurrant, strawberry, cherry, and raspberry) as well as commercial jams, made from the same fruits, were presented directly as standards.

*Discriminative testing method.* Triangular tests (NF EN ISO 4120, 2007) were performed by panel 1 using various aromatic reconstitution samples (**Table 1**), to evaluate the perception of different DMS concentrations in dilute alcohol solution and DRW containing the 12 esters.

In each test, three numbered samples were presented in random order: two identical and one different. Each judge used direct olfaction to identify the sample perceived as different in each test and gave an answer, even if s/he was not sure. Participants who recognized the different sample were then asked to describe the nuances in that sample, choosing a maximum of three

**Table 1. Olfactory impact of the addition of various concentrations of DMS to the complex fruity aromatic reconstitutions**

| | | $C_3C_2$ | $C_4C_2$ | $C_6C_2$ | $C_8C_2$ | 2Me $C_3C_2$ | 2Me $C_4C_2$ | 2OH $4MeC_5C_2$ | 3OH $C_4C_2$ | $C_2C_4$ | $C_2C_6$ | $C_2iC_4$ | $C_2iC_5$ | DMS | difference observed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **dilute alcohol solution** | | | | | | | | | | | | | | | |
| **Concentration (µg/L)** | ***Test 1*** | 150 | 200 | 200 | 200 | 250 | 50 | 400 | 300 | 10 | 2 | 50 | 250 | **5** | *** |
| | ***Test 2*** | 150 | 200 | 200 | 200 | 250 | 50 | 400 | 300 | 10 | 2 | 50 | 250 | **10** | *** |
| | ***Test 3*** | 150 | 200 | 200 | 200 | 250 | 50 | 400 | 300 | 10 | 2 | 50 | 250 | **20** | *** |
| | ***Test 4*** | 150 | 200 | 200 | 200 | 250 | 50 | 400 | 300 | 10 | 2 | 50 | 250 | **30** | *** |
| | ***Test 5*** | 150 | 200 | 200 | 200 | 250 | 50 | 400 | 300 | 10 | 2 | 50 | 250 | **50** | *** |
| | ***Test 6*** | 150 | 200 | 200 | 200 | 250 | 50 | 400 | 300 | 10 | 2 | 50 | 250 | **70** | *** |
| **dearomatized red wine** | | | | | | | | | | | | | | | |
| **Concentration (µg/L)** | ***Test 7*** | 150 | 200 | 200 | 200 | 250 | 50 | 400 | 300 | 10 | 2 | 50 | 250 | **5** | *** |
| | ***Test 8*** | 150 | 200 | 200 | 200 | 250 | 50 | 400 | 300 | 10 | 2 | 50 | 250 | **10** | *** |
| | ***Test 9*** | 150 | 200 | 200 | 200 | 250 | 50 | 400 | 300 | 10 | 2 | 50 | 250 | **20** | *** |
| | ***Test 10*** | 150 | 200 | 200 | 200 | 250 | 50 | 400 | 300 | 10 | 2 | 50 | 250 | **30** | *** |
| | ***Test 11*** | 150 | 200 | 200 | 200 | 250 | 50 | 400 | 300 | 10 | 2 | 50 | 250 | **50** | *** |
| | ***Test 12*** | 150 | 200 | 200 | 200 | 250 | 50 | 400 | 300 | 10 | 2 | 50 | 250 | **70** | *** |

*** 0.1% significant level; $C_3C_2$, ethyl propanoate; $C_4C_2$, ethyl butanoate; $C_6C_2$, ethyl hexanoate; $C_8C_2$, ethyl octanoate; $2MeC_3C_2$, ethyl 2-methylpropanoate; $2MeC_4C_2$, ethyl 2-methylbutanoate; $2OH4MeC_5C_2$, ethyl 2-hydroxy-4-methylpentanoate; $3OHC_4C_2$, ethyl 3-hydroxybutanoate; $C_2C_4$, butyl acetate; $C_2C_6$, hexyl acetate; $C_2iC_4$, 2-methylpropyl acetate; $C_2iC_5$, 3-methylbutyl acetate; DMS, dimethyl sulfide.*** 0.1% significant level; $C_3C_2$, ethyl propanoate; $C_4C_2$, ethyl butanoate; $C_6C_2$, ethyl hexanoate; $C_8C_2$, ethyl octanoate; $2MeC_3C_2$, ethyl 2-methylpropanoate; $2MeC_4C_2$, ethyl 2-methylbutanoate; $2OH4MeC_5C_2$, ethyl 2-hydroxy-4-methylpentanoate; $3OHC_4C_2$, ethyl 3-hydroxybutanoate; $C_2C_4$, butyl acetate; $C_2C_6$, hexyl acetate; $C_2iC_4$, 2-methylpropyl acetate; $C_2iC_5$, 3-methylbutyl acetate; DMS, dimethyl sulfide.

descriptors. The results of all the triangular tests were statistically analyzed, according to the tables given in the literature (Martin and de Revel, 1999; NF EN ISO 4120, 2007), based on the binomial law corresponding to the distribution of answers in this type of test.

Olfactory thresholds of DMS were determined in three sessions, by panel 1, using three different matrices, in a three-alternative, forced-choice presentation (3-AFC) (NF ISO 13301, 2002). Each test contained one positive sample supplemented with increasing concentrations of DMS (0.2, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5, 25, 50, and 100 µg/L) (**Table 2**). Each session consisted of 10 forced-choice tests. The first session used samples in hydroalcoholic solution, the second used dilute alcohol solution containing the 12 esters, and the third used DRW containing the 12 esters.

Specific «olfactory thresholds» of fruity mixtures were also established by panel 1. «Olfactory thresholds» of the fruity aromatic reconstitution (FAR) containing the 12 esters (fruity pool) were thus measured in two matrices: dilute alcohol solution and dilute alcohol solution containing 5 µg/L DMS (**Table 2**). «Olfactory thresholds» were determined in two sessions, in a 3-AFC presentation (NF ISO 13301, 2002). The samples in the first session consisted of 50 mL hydro-alcoholic solution, including one positive sample supplemented with increasing volumes of fruity pool (0.1, 0.2, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5, 25, and 50 mL). The second session consisted of the same 10 tests, using hydro-alcoholic solution supplemented with 5 µg/L DMS.

The results of all 3-AFC tests were statistically analyzed. The olfactory threshold was defined as the concentration where the probability of detection was 50%. This statistical value was determined using an adaptation of the ASTM-E1432 method (Cometto-Muñiz and Abraham, 2008). The concentration/response function is a psychometric function and fits a sigmoid curve ($y = 1/(1 + e^{(-\lambda x)})$). Detection probability was corrected using the chance factor ($P = (3*p - 1)/2$, where p = proportion of correct responses for each concentration and P = proportion corrected by the chance effect, 1/3 for 3-AFC). Sigma Plot 8 (SYSTAT) software was used for graphic resolution and ANOVA transform for nonlinear regression (Tempere *et al.*, 2011; Tempere *et al.*, 2012).

*Descriptive testing methods.* Descriptive analyses of DMS were carried out by panel 2 in dilute alcohol solution, using 10, 30, and 70 µg/L DMS. Judges were asked to choose a maximum of three descriptors. Descriptive analyses of FAR alone and supplemented with 5, 10, 20, 30, 50, and 70 µg/L DMS were also evaluated by the same panel.

Sensory profiles of aromatic reconstitutions were evaluated in two sessions by panel 1. The first session consisted of sensory profiles for black-berry fruit aroma intensity. The second session consisted of sensory profiles for blackcurrant, blackberry, strawberry, and raspberry aroma intensity. For each sample, the subject rated the intensity of these descriptors on a 100-mm scale ranging from «no odor perceived» on the left to «very intense» on the right (Martin and de Revel, 1999). The aromatic reconstitutions presented in these sessions are listed in **Table 3**.

*Statistical analysis.* Statistical data were analyzed using the Mann-Whitney statistical non-parametric test (XLSTAT software). The statistically significant level was 5% ($p<0.05$).

## RESULTS AND DISCUSSION

### 1. Odorant stimulus purity

GC-O analysis revealed parasite odors in some commercial products. These products were removed and new ones were purchased. Finally, all compounds

**Table 2. Composition of samples subjected to olfactory threshold determination in various matrices**

| Compound Tested | Tested Concentration (µg/L) | Matrix |
|---|---|---|
| DMS | 0.2 / 0.4 / 0.8 / 1.6 / 3.1 / 6.3 / 12.5 / 25 / 50 / 100 | dilute alcohol solution<br>FAR in dilute alcohol solution<br>FAR in DRW |
| **AR** | **AR diluted in 50 mL matrix (in mL)** | **Matrix** |
| FAR | 0.1 / 0.2 / 0.4 / 0.8 / 1.6 / 3.1 / 6.3 / 12.5 / 25 / 50 | dilute alcohol solution<br>dilute alcohol solution + 5µg/L DMS |

FAR, fruity aromatic reconstitution; DRW, dearomatized red wine; DMS, dimethyl sulfide.

**Table 3. Aromatic reconstitutions compared by sensory profiles**

| | Samples | | Descriptors |
|---|---|---|---|
| **1** | FAR in dilute alcohol solution | FAR in dilute alcohol solution **+ 5 µg/L DMS** | black-berry fruit, blackcurrant, blackberry, strawberry and raspberry |
| **2** | FAR in dilute alcohol solution | FAR in dilute alcohol solution **+ 10 µg/L DMS** | |
| **3** | FAR in dilute alcohol solution | FAR in dilute alcohol solution **+ 20 µg/L DMS** | |
| **4** | FAR in dilute alcohol solution | FAR in dilute alcohol solution **+ 30 µg/L DMS** | |
| **5** | FAR in dilute alcohol solution | FAR in dilute alcohol solution **+ 50 µg/L DMS** | |
| **6** | FAR in dilute alcohol solution | FAR in dilute alcohol solution **+ 70 µg/L DMS** | |
| **7** | FAR in DRW | FAR in DRW + **5 µg/L DMS** | blackcurrant, blackberry, strawberry and raspberry |
| **8** | FAR in DRW | FAR in DRW + **10 µg/L DMS** | |
| **9** | FAR in DRW | FAR in DRW + **20 µg/L DMS** | |
| **10** | FAR in DRW | FAR in DRW + **30 µg/L DMS** | |
| **11** | FAR in DRW | FAR in DRW + **50 µg/L DMS** | |
| **12** | FAR in DRW | FAR in DRW + **70 µg/L DMS** | |

FAR, fruity aromatic reconstitution; DRW, dearomatized red wine; DMS, dimethyl sulfide.

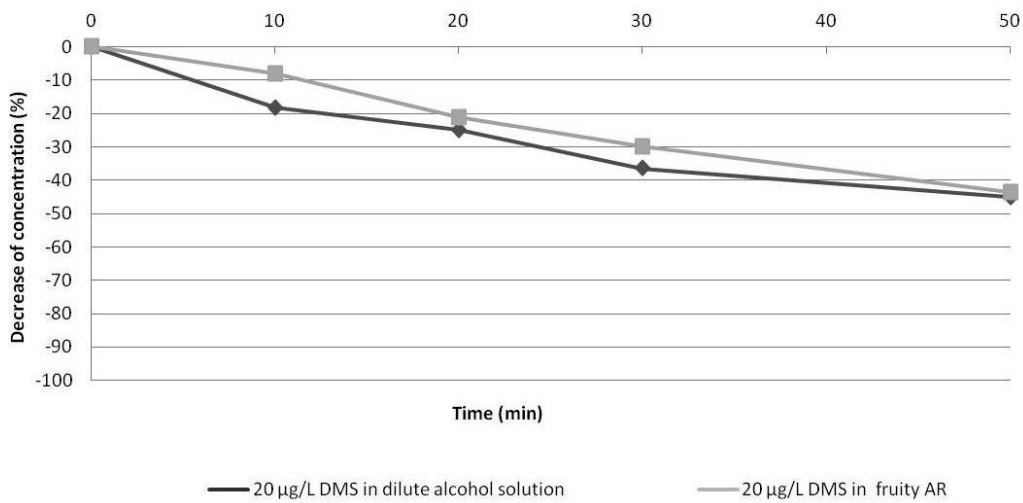


**Figure 1. Evolution of DMS concentrations in two different matrices - dilute alcohol solution and fruity aromatic reconstitution (AR) - during a sensory analysis session.**

used were effectively pure and any olfactory impurities were detected by the three judges who performed this analysis. Moreover, analytical methods (FID and FPD analysis) confirmed the high purity of the products, as specified by the manufacturers.

## 2. Evolution of sample composition during sensory analysis

Prior to the first sensory tests, the evolution of DMS was evaluated in two matrices, at concentrations of 20 µg/L, in order to assess the stability of the composition of the samples submitted to the panel. DMS remained stable for a few minutes in both matrices, with a decrease in concentration of less than 10% in the first 10 minutes (**Figure 1**). Similarly, Lytra *et al.* (2013) recently evaluated the evolution of the 12 esters, constituting the fruity pool, in dilute alcohol solution and demonstrated that most of these esters also remained stable for the first 10 minutes.

Consequently, the solutions presented to panel 1 were renewed every 10 minutes.

### 3. Direct organoleptic impact of DMS on quantitative odor perception

The addition test results are presented in **Table 1**. The addition of all DMS concentrations in both matrices - FAR in dilute alcohol solution and FAR in DRW - resulted in a statistically significant modification of the odor of the FAR. These results suggested that the DMS was present at suprathreshold concentrations and also showed that, at these levels, which are in agreement with oenological conditions, this compound contributed to the overall fruity aroma of the complex mixture (*tests* 1 to 12). As shown in **Figure 2**, the olfactory threshold of DMS was 1.74 µg/L in dilute alcohol solution, which was significantly lower (confidence interval CI <0.01) than that in dilute alcohol solution with FAR (2.80 µg/L) and DRW with FAR (3.67 µg/L), revealing a clear matrix effect. These findings justified the direct impact of this compound on aroma perception at the concentrations used in these reconstitution tests.

### 4. Indirect organoleptic impact of DMS on quantitative odor perception

The «olfactory threshold» of FAR was calculated in two different matrices. It was 1.80 mL in dilute alcohol solution and 0.55 mL in dilute alcohol solution containing 5 µg/L DMS (**Figure 3**) («olfactory threshold» expressed in mL fruity aromatic reconstitution (FAR) diluted in 50 mL matrix). The comparison between «olfactory thresholds» revealed that the «olfactory threshold» of FAR was 3.27 times higher ($p<0.01$) in dilute alcohol solution alone than when supplemented with 5 µg/L

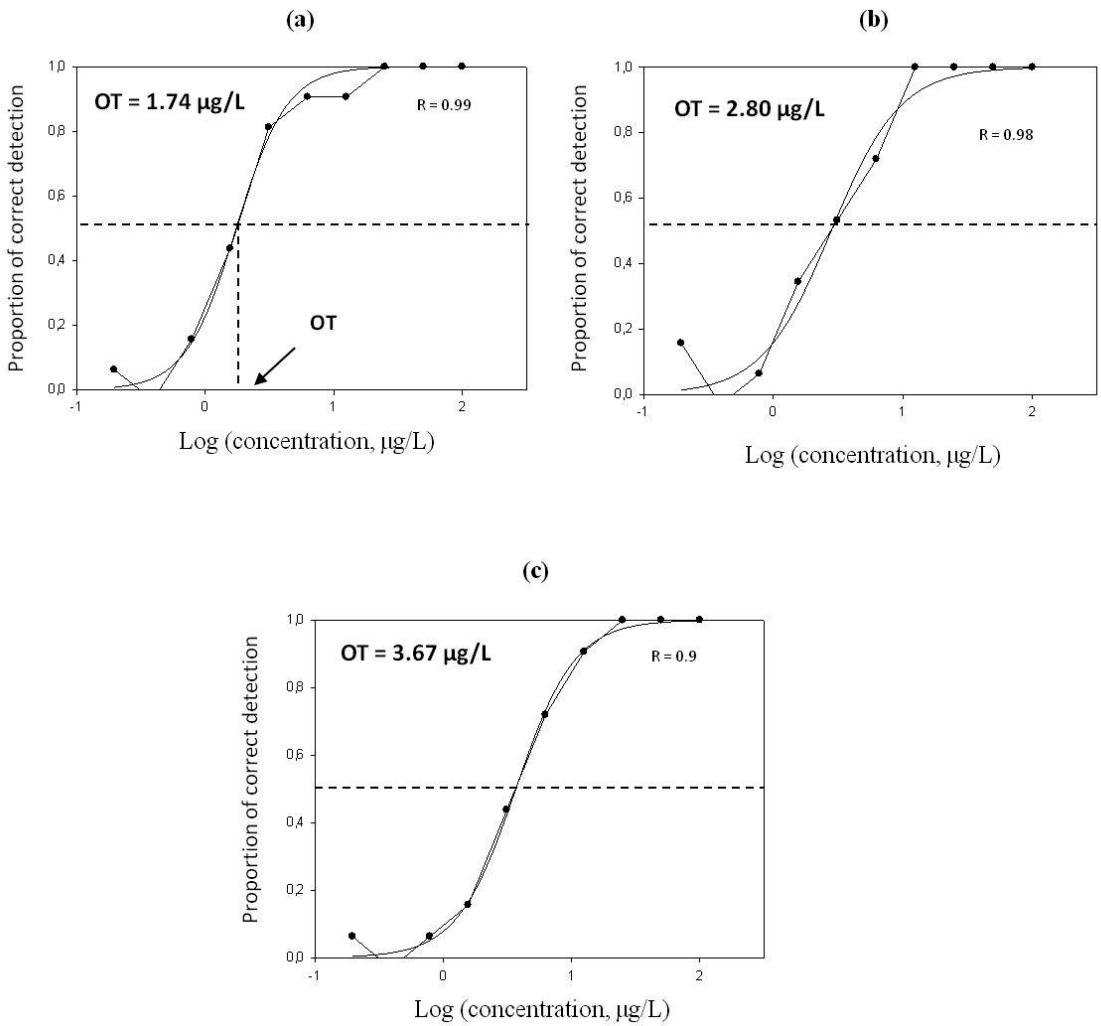


**Figure 2. DMS detection probability in three different matrices: (a) dilute alcohol solution, (b) FAR in dilute alcohol solution, and (c) FAR in DRW.**

OT, olfactory threshold; FAR, fruity aromatic reconstitution; DRW, dearomatized red wine; DMS, dimethyl sulfide. The curves are drawn according to a sigmoid function.

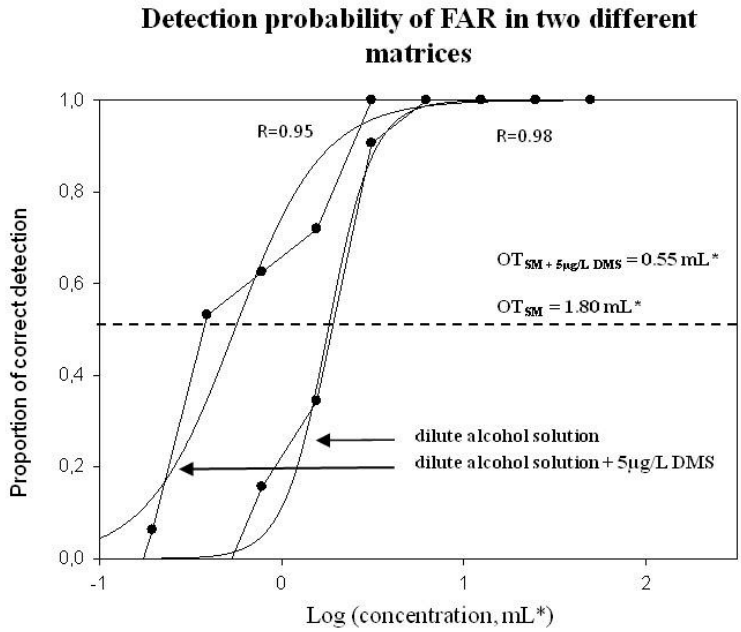


**Figure 3. Effect of DMS addition on the FAR detection probability.**
* expressed in mL fruity aromatic reconstitution (FAR) diluted in 50 mL matrix. MS, model wine solution (dilute alcohol solution); OT, olfactory threshold. The curves are drawn according to a sigmoid function.

DMS. These results demonstrated that DMS had an enhancing effect on the perception of the fruity pool. Segurel *et al.* (2004) evaluated the effect of DMS on the overall aroma of Grenache Noir and Syrah wines, using spiked with DMS to reach three levels (natural level, 100 µg/L, and 200 µg/L). The samples were then submitted to sensory descriptive analysis by direct olfactory evaluation, demonstrating that DMS levels near 100 µg/L had a major impact on the aroma of the Grenache and Syrah wines studied, conferring blackcurrant and strawberry/raspberry notes. It has also been demonstrated by Escudero *et al.* (2007) that while 10 µg/L of DMS was not directly perceived in a dearomatized wine, it conferred «sweet-fruity» or «green olive» notes to more complex mixtures (simultaneous addition of DMS, fruity esters, and norisoprenoids), accentuating the fruity notes and suggesting an enhancing effect. Antalick (2010) suggested that DMS was potentially able to intensify fruity aromas: in certain cases an intensification of fruity aromas accompanied the production of DMS during wine aging.

## 5. Organoleptic impact of DMS on qualitative odor perception

At concentrations perceived by the whole panel (10, 30, and 70 µg/L), DMS was mainly defined by sulfur descriptors and varied from an unspecific, pleasant light-sulfur odor for the lowest level to a strong-sulfur / «reduced» odor at higher concentrations.

The descriptive analyses, carried out by panel 2, demonstrated that the addition of 5, 10, 20, 30, 50, and 70 µg/L DMS to the FAR did not confer the specific DMS odor but led to black-berry fruit intensity increase. After these first observations, the samples were presented to panel 1.

Significant differences between FAR alone and supplemented with 5, 10, 20, 30, 50, and 70 µg/L DMS were highlighted for black-berry fruit intensity (**Figure 4a**) (first session). The average scores for black-berry fruit aromas were significantly higher for the FAR supplemented with DMS at all concentrations tested. These results confirmed the sensory importance of DMS, suggesting that it contributed actively to the black-berry nuances in the fruity matrix studied. To our surprise, regardless of the levels of DMS added (between 5 and 70 µg/L), the increase in black-berry fruit intensity remained the same.

During difference tests to assess the impact of DMS on the aroma of complex mixtures containing the fruity pool of esters, the descriptors most frequently cited by panel 1 were blackcurrant, blackberry, strawberry, and raspberry. These data are in agreement with Anocibar-Beloqui (1998), who established that levels of DMS in young Syrah and Xinomavro wines were correlated with «strawberry», «blackcurrant», and «raspberry» notes.

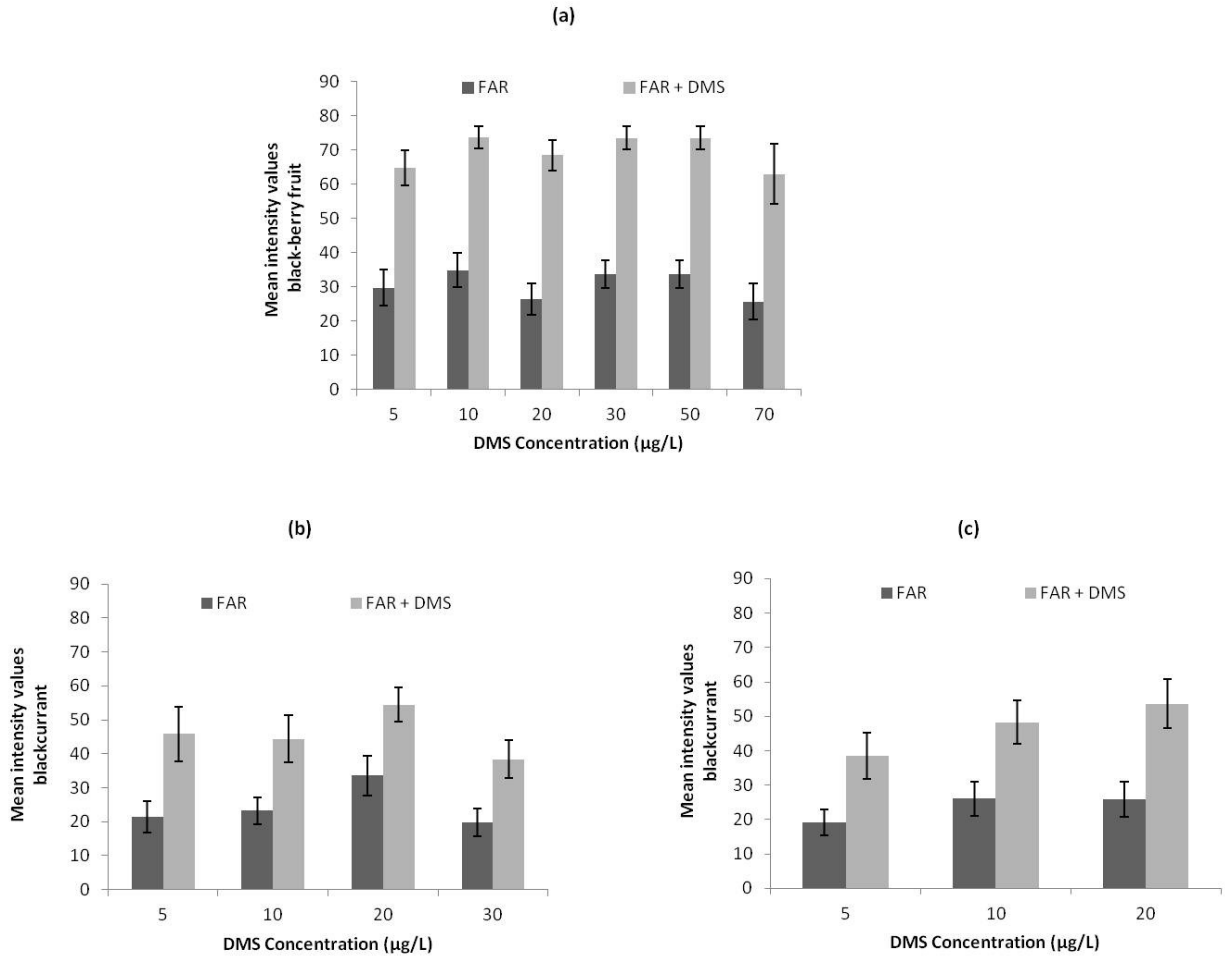


**Figure 4. Aromatic impact of various concentrations of DMS added to the complex fruity aromatic reconstitutions (FAR): (a) AR in dilute alcohol solution, (b) AR in dilute alcohol solution, and (c) AR in DRW.**

$p<0.05$. AR, aromatic reconstitution; DRW, dearomatized red wine; DMS, dimethyl sulfide.

The second sensory analysis session consisted of evaluating the most frequently used descriptors in AR: the average scores for blackberry, strawberry, and raspberry aroma intensity were identical after addition of DMS (results not shown), whereas blackcurrant aroma intensity was significantly higher for FAR in dilute alcohol solution supplemented with 5, 10, 20, and 30 µg/L DMS (**Figure 4b**) and FAR in DRW supplemented with 5, 10, and 20 µg/L DMS (**Figure 4c**), confirming the contribution of DMS to the blackcurrant nuances in these matrices. Rigou *et al.* (2014) recently demonstrated a correlation between thiol concentrations in red wines, such as 4-mercapto-4-methyl-2-pentanone (4MMP), 3-mercaptohexyl acetate (3MHA) and 3-mercapto-1-hexanol (3MH), and blackcurrant aroma intensity, suggesting that varietal thiols were potentially responsible for the development of blackcurrant aromas. This could suggest that compounds with relatively close chemical structures, such as DMS and thiols, may show similar sensory properties.

## CONCLUSION

Recent studies concerning complex mixtures provide evidence that any compositional modification, even of compounds present at levels well below their individual perception threshold, is likely to affect the olfactory characteristics of the mixture (Lytra *et al.*, 2013). The aroma of a mixture cannot, therefore, be considered simply as the sum of its components or the result of a single, dominant compound. On the contrary, all the components apparently have a potential impact on the overall aromatic character. Our data concerning DMS highlighted this effect. They revealed that this compound was present at levels above its perception threshold in dilute alcohol solution, as well as in more complex matrices, from an olfactory and chemical point of view, such as

aromatic reconstitutions of fruity fractions. The findings clearly indicated that DMS played a direct role in the overall aroma of the matrices studied. However, DMS cannot be considered a «key» compound in fruity aroma, since it does not present fruity aromas. In contrast, its indirect contribution to fruity aroma expression was confirmed: in dilute alcohol solution, the presence of DMS at low levels resulted in a decrease in the «olfactory threshold» for the fruity pool, reflecting its quantitative contribution to overall fruity aroma intensity. At the concentrations used in these tests, DMS produced an enhancing effect, increasing the perception of fruity character.

This work also revealed the indirect qualitative impact of DMS on fruity aroma expression. Although it does not present any fruity aromas, it was an active contributor to black-berry fruit and, specifically, blackcurrant nuances. The intensity of these aromas was significantly higher when this compound was added, reflecting its qualitative contribution to overall aroma intensity. This research highlighted the role of DMS as a natural enhancer of these aromas in complex fruity mixtures.

The typical aroma of red wines from *Vitis vinifera* cv Merlot noir and Cabernet Sauvignon is formed during alcoholic fermentation and evolves during the first years of aging. Contrary to most ethyl esters produced during alcoholic fermentation, the levels of branched esters increase during aging. This behavior parallels that of DMS during the first years of aging, suggesting that these compounds enhance the typical fruity aromatic character. Moreover, the levels of DMS still increase during wine aging. Its implication in the molecular composition of wine bouquet and in the complexification and intensification of wine aroma during aging becomes a strong hypothesis.

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