JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Role of 3-Methyl-2,4-nonanedione in the Flavor of Aged Red Wines

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ABSTRACT: GC-MS in chemical ionization mode (CI) was used as a simple, sensitive method for assaying 3-methyl-2,4-nonanedione (MND) in 67 red wines. MND content was shown to be lower in nonoxidized red wines and higher in oxidized red wines, that is, systematically exceeding the perception threshold (62 ng/L). Concentrations up to 340 ng/L in the most oxidized red wines were also evidenced. According to these quantitative data, the presence of MND alone was shown to modify significantly the flavor of the red wine as evaluated from fresh fruit flavor in red wine without MND to aromatic expression of rancio in wines with elevated concentrations (308.9 ng/L). Incidence of oxygen on its formation was also investigated. For the first time, elevated concentrations of this compound in various wines were demonstrated, that is, white, rosé, botrytized wines, and fortified wines made with over-ripened grapes. The lowest levels (2.9 ng/L) were found in nonoxidized white wines, whereas the highest levels were found in oxidized botrytized wines (293.8 ng/L). These results tend to demonstrate that MND is in general a good marker of oxidation in wines made with a maceration step between the skin and the pulp. These preliminary results provide a new analytical explanation of what is known in enology as the "vin de garde" concept.

KEYWORDS: *3-methyl-2,4-nonanedione, wines, aging, oxygen, premox, flavor, oxidation, aging potential*

INTRODUCTION

The reputation of famous red wines is strongly associated with their aging potential. These wines retain the flavor nuances of young wines while developing specific varietal nuances. This kind of aging results in what is called a "bouquet" of reduction. Although this specific organoleptic character is highly prized by connoisseurs, this ideal sort of aging does not occur in every wine. Most of the time, wine flavor develops quickly, resulting in a loss of complexity and personality, that is, the flavor found in every oxidized red wine.

When wines age under continuous oxidative conditions, the evolution of red wine flavor can be summarized in four steps. The first is the loss of fresh fruit flavor, followed by the development of aromatic nuances reminiscent of prune and fig. With regard to flavor formation, these two steps constitute what is known as premature aging or "premox". In our experience of tasting red wines, the presence of these over-riding odors affects the quality and subtlety of wine flavor and may shorten its shelf life. The flavors found in the third and fourth steps, rancio and turpentine, are traditionally and systematically associated with the "end of the road" for old red wines.

Prune and fig nuances are often found in old sweet fortified,[1] Madeira wines,[2] and Port wines.[3] In these old wines high concentrations of sotolon (0.1−1 mg/L) may explain the desired prune nuances. To our knowledge, such high concentrations are not usual in old red wines, in which they remain below sotolon's perception threshold.[8] More recently, some studies revealed the existence of interaction (additive or synergic) between some oxidation-related aldehydes such as (*E*)-2-alkenal and aliphatic aldehydes producing these flavors.[4] Another interesting aldehyde is methional. This well-known potato-smelling compound was identified first in oxidized white wine.[5] Addition of this compound in a red wine is able to transform the fresh fruit note into a dry fruit note. According to the authors this effect is particularly strong in the presence of norisoprenoids.[6]

We recently identified 3-methyl-2,4-nonanedione (MND) in prematurely aged red wines marked by an intense prune flavor.[7] MND is a volatile compound with an intense odor of anise and dried parsley.[8−10] Its exact contribution to the flavor of oxidized red wines has remained elusive to date, and its formation pathway during aging has not previously been investigated. This paper reports an assay of MND in red wines according to the vintage, appellation, and its contribution to the prune and oxidative flavor of these wines. We extended our assays to other wines: white, rosé, botrytized, over-ripened, and fortified. In addition, the impact of dissolved oxygen on its concentrations during the aging of a red wine was also studied.

MATERIALS AND METHODS

Chemicals. 3-Octanol, BHA (2 + 3-*tert*-butyl-4-methoxyphenol isomers), and ethylenediaminetetraacetic acid sodium salt (EDTA, 100%) all came from Sigma-Aldrich (St. Quentin Fallavier, France). Anhydrous sodium sulfate (99%), pentane, dichloromethane (Rectapur grade), and methanol (HPLC grade) were supplied by Prolabo (France). 3-Methyl-2,4-nonanedione (>99%) came from Chemos GmbH (Germany).

Wine Samples. One hundred and twenty bottles of wine were of different appellation, color, winemaking technique, and vintage as described in Table 1. The samples were selected on the basis of the visual quality of the cork stopper. Leaking bottles were not included in the study. Wines with clear cork taint were also excluded. Each bottle was tasted and analyzed in 2009. The wines selected for studying the evolution of 3-methyl-2,4-nonanedione in the great wine and the second wine were from the same estate in the Pessac-Leognan

Received: January 30, 2013
Revised: June 21, 2013
Accepted: June 25, 2013
Published: July 17, 2013

dx.doi.org/10.1021/jf400348h | J. Agric. Food Chem. 2013, 61, 7373−7380

Table 1. Characteristics and Distribution of Wines According to Their Color, Origin, and Vintage (n = 120)

wine	code	appellations	grapes	vintages	quantity
red	R	Pessac-Léognan Margaux, Pomerol, Bordeaux, Château Neuf du Pape, Rioja	Merlot, Cabernet Sauvignon, Cabernet Franc, Grenache, Tempranillo	1981–2008	67
rosé	Ro	Côte de Provence, Clairet	Grenache, Merlot	1998–2008	10
white	W	Bordeaux, Pessac-Léognan	Sauvignon, Semillon	2001–2008	10
white, over-ripened	Wo	Jurançon, Alsace	Gerwürstraminer Petit Manseng	1990–2005	8
white, botrytized	Bot	Sauternes, Barsac, Loupiac	Semillon, Sauvignon	1929–2007	17
red, fortified	RF	Banyuls, Maury	Grenache	1999–2008	8

appellation. These red wines were made by blending Merlot and Cabernet Sauvignon wines produced with standard winemaking procedures, including contact with oak wood. The closure was a top-grade natural cork stopper and came from the same batch for the two wines. Bottling was performed in the same conditions for the two wines. Wines were aged in the same conditions in a cellar kept at 16 °C during the experiment. For each vintage from 2002 to 2006, three bottles of both wines were analyzed in 2009.

Red wine for oxidation experiments and MND addition experiments was nonwooded Bordeaux wine made by blending Merlot and Cabernet Sauvignon wines from the 2009 vintage.

Extraction Procedure. The entire method was described in ref 11. Briefly, wine samples (100 mL) were spiked with 100 μL of 3-octanol (100 mg/L, EtOH) as an internal standard, 100 μL of BHA (100 g/L, EtOH), and 100 μL of EDTA (60 g/L, H_2O). The wines were extracted three times with 10, 5, and 5 mL of pentane/dichloromethane (1:1, v/v) solvent mixture by magnetic stirring (10, 5, 5 min; 750 rpm). The three organic phases obtained after each extraction were blended, dried over anhydrous sodium sulfate, and concentrated to 0.5 mL under a nitrogen stream. The vessel was shaded to prevent photo-oxidation phenomena during extraction and concentration.

Gas Chromatography–Mass Spectrometry (GC-MS) Conditions. A CP3800 gas chromatograph coupled with a 4000 ion-trap mass spectrometer from Varian was used to analyze the organic extract. The gas chromatograph system was equipped with a split/splitless 1078 programmable-temperature injector and a fused-silica capillary column 60 m long, with a 0.25 mm internal diameter, coated with a 0.5 μm thick film of polyethylene glycol (BP20, SGE, France). The carrier gas was He (Air Liquide, Bordeaux), Alphagaz 2 grade, with a flow rate of 1 mL/min. One microliter of sample was injected via the autosampler (Combipal, CTC Analytics). The injector was set to 230 °C in splitless mode (closure time, 0.8 min) with a 50 mL/min split flow. Oven temperature was initially set at 40 °C for 1 min, then raised to 190 °C at 3 °C/min and to 240 °C at 15 °C/min, and kept at that temperature for a further 20 min. The transfer line and manifold were maintained at 230 and 50 °C, respectively. Trap temperature was maintained at 150 °C. For positive chemical ionization with methanol, the liquid reagent in gaseous phase was introduced into the ion trap through the standard inlet and its pressure regulated so that the total ion current of the reagent was close to 13000 counts, with an ionization time close to 100 μs. The CI liquid valve was opened 2 min before the retention time of the analyte to allow the reagent gas to stabilize. The acquisition (CI mode) was divided into two segments: first segment, 3-octanol (28–31 min, full scan, m/z 71); second segment, MND (42.5–45 min, μSIS, m/z 171).

Sensory Experimental Procedure. Sensory analyses were performed by a panel of 12 judges recruited from the staff of the research unit. All panelists had extensive experience in wine-tasting and participated regularly in sensory panels with red Bordeaux wines. All of the assessments were performed at room temperature (18 ± 1 °C) in individual booths under daylight lighting. Fifty milliliters of wine was presented in standard ISO 3591 XL5-type tasting glasses with glass covers identified by three-digit random codes and assessed within 15 min of pouring. Each wine was submitted to the panelists just after the bottle was opened. They were asked to evaluate the intensity of prune flavor and oxidation flavor on a 0–5 scale (0, no odor; 1, discrete odor; 2, odor just perceived; 3, odor recognized; 4, clear odor; 5, strong odor).

MND Addition Experimentation. Before performing the sensory experiment, we assayed MND in the wine to check that it was at a very low concentration (8.9 ng/L). The experiment consisted of six different concentrations of MND selected to cover the concentration range found in red wines, the concentrations being presented in ascending order (0, 50, 90, 170, 250, 330 ng/L). As MND is a very reactive compound in red wines,[11] we decided to spike it in the red wine sample (100 mL) just before beginning sensory evaluation. For each sample with increased concentrations of MND, the panelists had to assess respectively fresh fruit, anise, minty, fruit pit, prune, and rancio odor intensities on a 0–5 scale. During the sensory session, six samples, one per MND concentration, were presented to each panelist.

Determination of Perception Threshold. The perception threshold of MND in model solution (12% ethanol, 5 g/L tartaric acid, pH 3.5) and wines was determined by triangular tests. The solutions were presented in glasses corresponding to Association Française des Normes (AFNOR) standards. The odor perception threshold corresponded to the minimum concentration below which 50% of 26 tasters (all from the Faculty of Enology) statistically failed to recognize the difference from the control.

Oxidation Experiment. Two batches of a nonwooded red wine (1 year old) sample were stored in 200 mL bottles with a 10 mL head space saturated with nitrogen and stored at room temperature in a dark place. This experiment was done in duplicate. All of the measurements of dissolved oxygen in wine were performed using an electrochemical probe (Orbisphere Geneva, Switzerland). In the first experiment, the control wine was kept without oxygen ($[O_2] < 20\ \mu g/L$). In the other batch, we used synthetic air (Air Liquide, France) to adjust the dissolved oxygen concentration to 6 mg/L. The experiment was conducted over 8 days. Throughout the experiment, samples were opened (at 1, 2, 4, and 8 days) under nitrogen atmosphere after 3 min of stirring, and an aliquot of sample was taken for GC-MS measurement.

Statistical Analysis. In each case, data were subjected to a repeated-measures analysis of variance (ANOVA) to determine if differences between treatments were significant. Duncan least significant differences (LSDs) were calculated to compare differences between means (following significance in the ANOVA). All effects were evaluated at the 5% level unless otherwise stated. We also performed a nonparametric test like the Kruskal–Wallis test when necessary. These tests were performed with Spad software (Coheris, Paris, France).

RESULTS AND DISCUSSION

MND Distribution in Red Wines. MND was first identified in oxidized red wines marked by prune flavors.[11] Using a previously validated method, MND was assayed in 67 red wines samples from 6 appellations and 4 types of grapes (Table 1). Wines were also selected according to their age, from very young wines (1 year old) to 28 years old. The wines were classified by the jury according to the intensity of their oxidative flavors (0–5 scale) and characterized as either being nonoxidized wines (0–<2) or oxidized wines (≥2–5). The distribution of MND concentrations of the wines according to this scale is reported Figure 1.

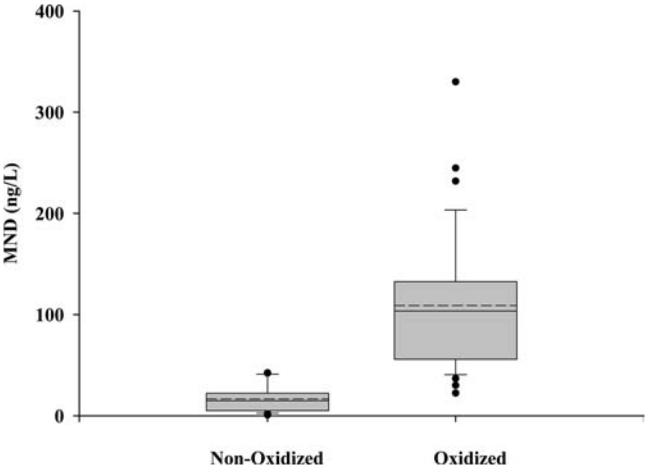

Figure 1. Box−whisker plot of MND concentration in nonoxidized red wines (oxidation intensity <2) and oxidized red wines (oxidation intensity ≥2). The midline of the box represents the median value (solid) and average value (short dash); the upper and lower bounds of the box represent the interquartile range (IQR). Values that are more than 1.5 times the length of the box are represented as dots. n = 67.

We observed first that the amount of MND in red wines varied considerably in the different types of wine and ranged from 4.2 to 340 ng/L. The MND contents of the oxidized red wines were clearly higher than those of the nonoxidized wines ($p < 0.01$) and ranged from 1 to 21 times the perception threshold (16 ng/L). In particular, there was a significant linear correlation (data not shown) between the intensity of oxidation flavor and the MND level in these wines ($R^2 = 0.510$, $p < 0.01$).

In view of these preliminary data, we also examined a specific nuance found in every oxidized red wine: the prune nuance. As depicted in Figure 2, the relationship between the average prune flavor intensity obtained for each of the wines and its MND content was linear and significantly correlated (R^2 = 0.6081, $p < 0.0001$, α = 5%). Wines with no prune character (average 0) contained an average value of 14 ng/L MND. Those considered as having a very weak prune flavor (level 1) had an average value of 41 ng/L, and above that value, the perception of this aromatic character increased progressively.

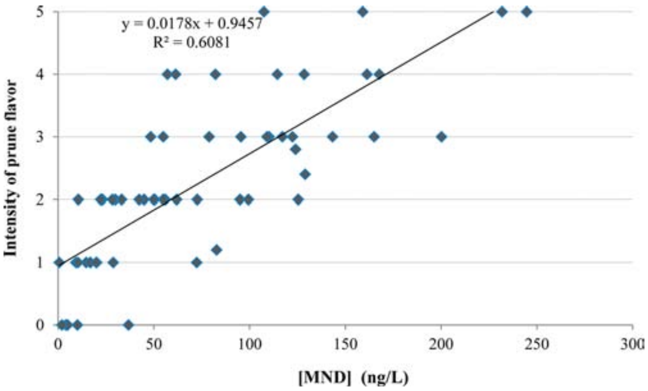

Figure 2. Correlation between average prune flavor intensity obtained for each of the 67 red wines and MND concentration determined by GC-MS analysis.

Empirically, it is well-known that the older the wine, the more it is thought to be marked by oxidation flavors. Surprisingly, global analysis of the data did not show any relationship between age and MND level (R^2 = 0.112) (data not shown). As the capacity of a wine to age depends on many factors including environmental factors in the vineyard, wine-processing techniques, quality of the stopper, and temperature of conservation during bottle aging, high MND levels can be found not only in old wines but also in younger ones (<5 years).

MND Distribution in Other Wines. Winemakers and enologists know that there are other wines such as sweet fortified wines,[12,13] clarets, and botrytized wines in which this aromatic nuance depends on the level of their perceived oxidation intensity. Therefore, to gain greater insight into the organoleptic contribution of MND in these different wines, we decided to explore systematically the organoleptic impact of MND in wines. Hence, our selection covered a wide range of the types of wines commercially available. The MND content was assayed not only in wines from different origins and vintages but also in those from different winemaking processes, including wines made with white grapes harvested at maturity (dry white wine), at the over-ripened stage (sweet over-ripened wines) and botrytized (botrytized white wine), rosé wines, and sweet fortified wines (Table 1). Before each analysis, all of the wines were assessed by a training panel and chosen according to the intensity of their oxidation flavors. Consequently, the descriptors currently used to describe the flavors of oxidized wines are very different according to the type of wines, ranging from honey, beeswax nuances for white wines to dried prune for red wines, and nuts and rancio for fortified wines. Hence, we chose the term "oxidation" as a nonspecific descriptor for describing the global flavor of all the selected wines. The wines were classified according to the intensity of their oxidative flavors. With an average score of <2, the wine was considered to be not oxidized (NOx) and was held to be oxidized (Ox) with a score of ≥2. Wines made under reducing conditions (compared to traditional oxidative winemaking and aging conditions found for Port and sweet fortified wines) were considered to have nontypical oxidative aging flavors, whereas these aromas were normally associated with wines made under "traditional" oxidizing conditions as sweet fortified wines.

For nonoxidized wines, the MND concentrations varied from one wine to another. The lowest concentrations were found in dry white wines (W). Nonoxidized botrytized wines reached the highest levels at 60.1 ng/L (Table 2). For all of the wines analyzed, the concentration of MND was mostly lower than or close to the perception threshold (16 ng/L for dry wines, 97 ng/L for sweet wines). The sweet fortified wines did not appear in this list as we considered them as being systematically oxidized. On the contrary, the MND content of all the oxidized wines (except white wines) seemed systematically higher, reaching 293.8 ng/L for oxidized white botrytized wine.

For each type of wine, data distribution was checked using the Shapiro−Wilk test at a significance level of α = 0.05. A relatively high degree of deviation from normality was observed. Thus, we attempted to differentiate the wines according to their oxidation aroma and the type of wine by applying the nonparametric Kruskal−Wallis test to the MND variable. Each family of wines was compared for each modality (N-Ox and Ox). The Kruskal−Wallis test applied to the five groups of wines according to the modality showed a highly significant difference between oxidized and nonoxidized wines

Table 2. MND Content in Nonoxidized (N-Ox) and Oxidized (Ox) Samples from 120 Different Wines

	N-Ox (ng/L)		Ox (ng/L)	
	min−max	av (SD)[a]	min−max	av (SD)[a]
red ***[b]	1.9−42.4	16.6 (12.9)	22.3−330.1	108.9 (65.4)
white	2.9−9.2	7.1 (3.8)	8.7−23.1	14.5 (5.5)
white, over-ripened ***	2.8−14.0	9.3 (4.5)	17.9−65.2	46.9 (18.4)
white, botrytized ***	7.7−60.1	40.9 (20.1)	40.6−293.8	152.1 (76.1)
rose ***	2.8−10.5	6.2 (2.2)	51.6−103.6	74.91 (19.4)
red, fortified wines ***			60.2−191.3	115.2 (39.6)

[a] Values in parentheses correspond to standard variation of group. Significant for factor "oxidation" according to Kruskal−Wallis test. [b] ***, significance with $p < 0.001$.

($p < 0.01$). However, there was an exception with white wines, which did not present any significant differences in their MND levels according to the intensity of oxidation flavors.

Determination of 3-Methyl-2,4-nonanedione Perception Threshold. The preliminary study sought to identify a compound associated with the prune flavor of prematurely aged red wines. In that study, the detection threshold of MND in a model solution was found to be 16 ng/L, but the organoleptic impact of MND in red wines could not been clearly determined.[7] Therefore, the first objective of the present study was to analyze the organoleptic impact of MND in red wines. Nevertheless, we included many wines from different origins to gain initial insight into the organoleptic role of MND in wines. For this reason, we included the perception threshold of MND in a botrytized wine model solution (Table 3).

Table 3. Perception Threshold of MND in Several Matrices

	perception threshold (ng/L)
must model solution[a]	62
dry wine model solution[b]	16
botrytized wine model solution[c]	97
red wine	59
red wine[d]	62

[a] G, 120 g/L; F, 120 g/L; tartaric acid (5 g/L), pH 3.5. [b] EtOH (12% vol), tartaric acid (5 g/L), pH 3.5; see ref 7. [c] EtOH (14% vol), G+F (5% vol potential), tartaric acid (5 g/L), pH 3.5. [d] Detection threshold calculated from the assay of MND and the sensorial evaluation of prune intensity from 67 wines.

The perception threshold in wine is only an indicative value owing to the large variations in composition from one wine to another. However, it is useful for estimating the concentration above which the odor produced by the compound under investigation is detected.[14] Because such measurement is closely linked with the wine matrix and creates considerable diversity and complexity in wines, we followed the procedure used by Roujou de Boubee[15] to determine the 2-methoxy-3-isobutylpyrazine (IBMP) detection threshold in a wine matrix. Sixty-seven red wines from different appellations, grapes, and vintages were ranked on a 0−5 scale according to the intensity of their prune flavor. We have found that the threshold of MND beyond which the red wine was marked by prune character was 62 ng/L. Classical determination of the MND detection threshold by directional triangular tests of five increasing concentrations in a young nonoxidized red wine gave similar results (Table 3).

The level of detection of MND in a botrytized white wine model solution was 5-fold higher than its perception threshold in dry red wine, partly due to the impact of ethanol concentration. The determination of its perception threshold in a must model solution containing a high carbohydrate level (glucose + fructose = 220 g/L) showed an unexpected increase in the perception threshold. This "salting in" effect of sugar was already observed for volatile compounds with an elution temperature by gas chromatography fitted with a wax column higher than 92 °C, which is the case for MND.[16] Changes in threshold values are thought to arise from changes in the headspace partition coefficient of a compound as a result of either a change in solubility or an interaction with other solute components.[17] De Roos and Wolswinkel found that the addition of sucrose increased the hydrophobic character of the solution, thereby modifying the intrinsic property of the solvent.[18] In our particular case, MND was present as a keto−enol tautomerism affecting its hydrophobicity and extraction behavior. Hence, it cannot be ruled out that the high carbohydrate level modified the equilibrium of MND to a higher soluble form in water such as keto−enol.

Sensory Analysis. *Organoleptic Impact of MND in Red Wine.* The first step in the sensory analysis was to select a wine on the basis of these organoleptic properties. The flavor of the red wine had to be marked by fresh fruity flavors and had to be nonwooded and nonoxidized. Six descriptors were chosen on the basis of their association with MND flavor and oxidized red wine flavor. As previously described,[7] MND evokes various flavors such as minty, fruit pit, or anise, according to its concentration when added alone to a red wine model solution. The terms prune, fruit pit, and rancio have often been used to describe specifically red wine that tends to be oxidized or clearly oxidized. To throw light on the contribution of MND to red wine flavor, each panelist's means and standard deviations were compared for each MND concentration. A one-way ANOVA and a multiple comparison of means by the Duncan test were performed for each descriptor.

As depicted in Figure 3, increasing the concentration of MND in a red wine highly modified its flavor, even at low concentrations. Among the six odor description terms selected, only fresh fruit, prune, fruit pit, and rancio nuances showed highly significant differences ($p < 0.001$) among the supplemented samples. We showed that the samples could be considered as perceptually different on the basis of the intensity of these descriptors.

As expected, addition of MND to a fresh fruity red wine removed its "fresh fruit" nuances and enhanced specific nuances reminiscent of dried fruit. For the descriptor fresh fruity, the first concentration (50 ng/L) was significantly different from that of the nonsupplemented wine. The base wine contained MND at trace level (8.9 ng/L). Therefore, the first concentration reached a value close to its perception threshold in red wine. Moreover, there was a significant negative correlation between the MND concentration and the intensity of fresh fruit flavor (Table 4), so we hypothesized that its presence at a low level acted as a mask rather than directly making an organoleptic contribution to the wine flavor. For concentrations reaching 250 and 330 ng/L, this nuance was no longer detected by our panelists (Figure 3).

Minty and anise nuances were detected in the control wine. These descriptors are not common in young Bordeaux red

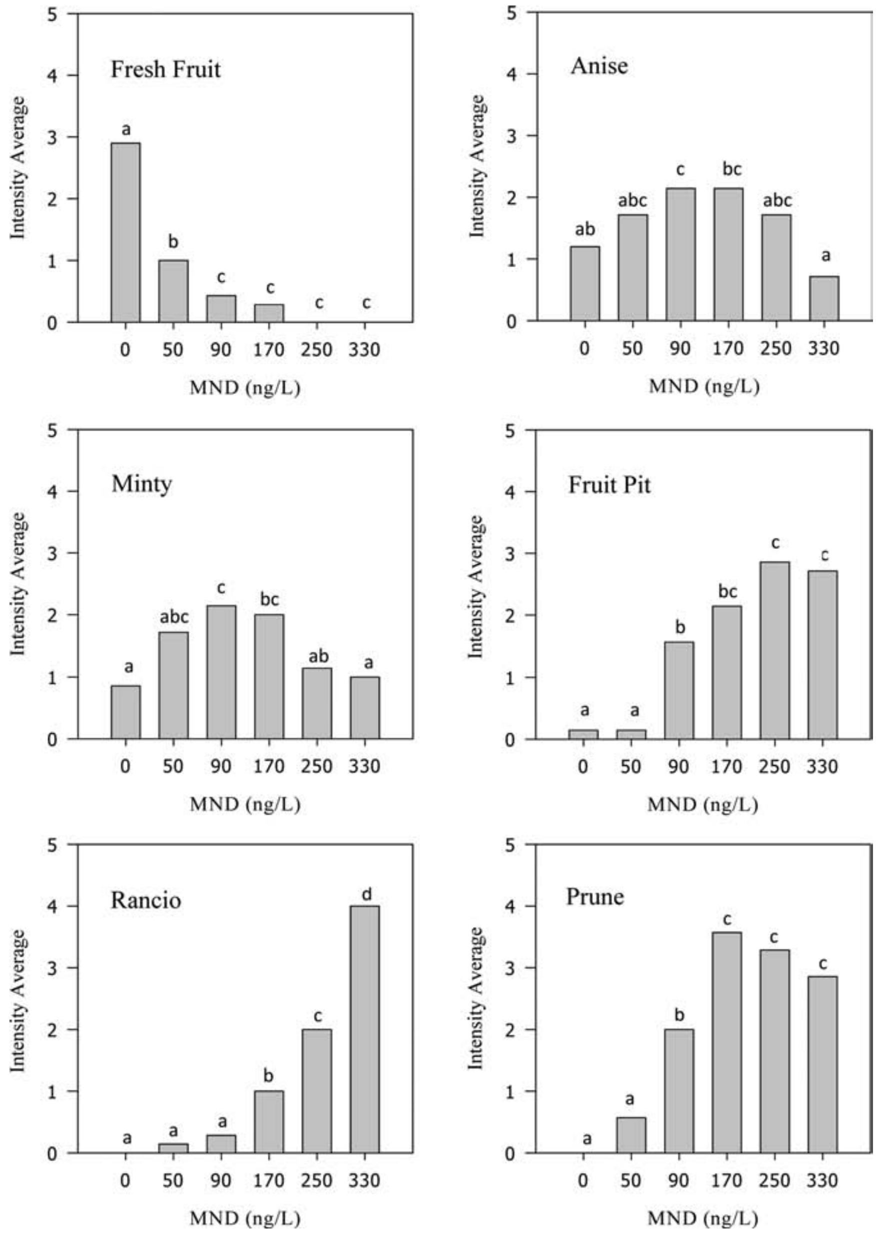

Figure 3. Means of the evolution of the odor intensity of several descriptors according to increasing MND concentrations in red wine. Different letters indicate significantly different values by Duncan's test at (p < 0.05).

Table 4. Correlations between MND Concentrations and Selected Descriptors

descriptor	correlation coefficient[a]
fresh fruit	−0.720 **
anise	−0.066 ns
minty	−0.108 ns
prune	0.675 **
fruit pit	0.740 **
rancio	0.869 **

[a] Significance level at $p < 0.01$; ns, nonsignificant.

wines, but in this particular case they might have been associated with the freshness of the wine. With this pair of descriptors, the intensity of their perception slightly increased with increasing concentrations of MND. At intermediate levels (50−90 ng/L), MND was found to modify the perception of these nuances in a red wine ($p < 0.01$), whereas at 90 ng/L these descriptors seemed to be more intense (Table 4). On the contrary, at a higher concentration, they were significantly less intense. Hence, the intensity of these descriptors is not clearly correlated with increasing MND levels (Table 4).

Fruit pit, prune, and rancio flavors were not detected in the control wine. According to its concentration, MND contributed significantly several typical nuances ($p < 0.001$) to red wine. Moreover, characteristic descriptors of oxidized red wines such as prune, fruit pit, and rancio were highly correlated with positive correlation coefficients of 0.675, 0.740, and 0.869, respectively. A concentration of 170 ng/L, which is greater than its perception threshold, imparted over-riding prune and fruit pit flavors. At very high MND levels ranging from 170 to 330 ng/L, rancio and prune flavors dominated the overall flavor (Table 5). Finally, MND was found to modify deeply the flavor of the wine according to its concentration. Such a broad range of flavors associated with MND has not been previously

Table 5. Evolution of Organoleptic Attributes of a Young Red Wine According to Increasing Concentrations of MND[a]

[MND] (ng/L)	fresh fruit	anise	minty	prune	fruit pit	rancio
0 **[b]	2.92 (0.78) d	0.71 (0.75) bc	0.85 (0.69) c	0.00 (0.00) a	0.14 (0.37) ab	0.00 (0.00) a
50 **	1.00 (0.53) b	1.71 (0.95) c	1.71 (0.75) c	0.14 (0.38) a	0.14 (0.38) a	0.14 (0.38) a
90 **	0.42 (0.49) ab	2.14 (0.78) bc	2.14 (1.00) bc	2.00 (1.34) abc	1.57 (0.69) abc	0.28 (0.53) a
170 **	0.28 (0.48) a	2.14 (1.06) c	2.00 (1.15) bc	3.57 (1.13) d	2.14 (0.69) c	1.00 (0.82) ab
250 **	0.00 (0.00) a	1.71 (1.2) b	1.14 (0.9) b	3.28 (0.75) d	2.85 (1.25) cd	2.00 (0.81) bc
330 **	0.00 (0.00) a	0.71 (0.9) a	1.00 (0.9) a	2.85 (0.9) b	2.71 (1.38) b	4.00 (1.00) c

[a] Values in parentheses correspond to standard deviation for the group. In the same row, means with a common letter are not significantly different $p < 0.05$ (Duncan's test). Significance of factor "organoleptic characteristic" according to one-way ANOVA. [b] ** indicates significance with $p < 0.01$.

documented. Of course, the contribution of MND to the flavor of various food products has already been reported. It contributes to the hay-like off-flavor induced during the drying process of dry parsley[9] and dry spinach,[10] to the off-flavor of reversed soybean oil,[8] and to the light-induced off-flavor of butter and butter oil.[19] In these cases, its formation during processing or storage is always associated with an impairment of the organoleptic quality of the product. On the contrary, MND can also contribute to the complexity and the typical characteristic flavor of green tea.[20,21] The concentration of MND found in these products ranged from 10 to 90 μg/kg in dry parsley[9] and dry spinach[10] to a few mg/kg in soybean oil.[22,23] Hence, according to the type of food, MND either is a marker of intrinsic organoleptic quality or has a negative sensorial effect.

Role of Oxygen in the Formation of MND in Red Wines. The matrix used in this study was a nonwooded commercial red Bordeaux wine. The oxygen was dissolved in the wine, and samples were kept without additional oxygen ingress until the end of the experiment. The MND level in the red wine was low (26 ng/L) and below its perception threshold in wines (62 ng/L). Incidence of oxygen on the formation of MND is reported in Figure 4. In the wine supplemented with oxygen, there was an increase in MND concentration compared to its level in the red wine kept without oxygen. During chemical oxidation of a red wine, this evolution took place after a lag time of at least 2 days concomitant to oxygen consumption. At the end of the experiment, the level of MND had reached 80 ng/L, a value higher than its perception threshold. Therefore, the role of the oxygen and oxidation mechanisms on the formation of MND in red wines seemed to be important.

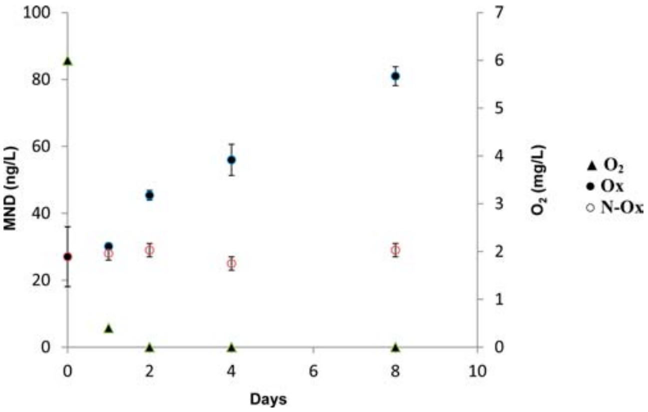

Figure 4. Evolution of MND and oxygen levels in a red wine stored with (Ox) or without oxygen (N-Ox) ($n = 2$).

Incidence of Sulfur Dioxide (SO_2). The presence of SO_2 in wine has a marked incidence on wine aging. Its concentration gradually decreases during storage, a mechanism that is now well understood. It also plays another important role in wine by forming adducts with carbonyl compounds, thereby increasing their odor threshold. Although some SO_2 is produced naturally in wine owing to yeast metabolism, it is added during winemaking for protective purposes. Therefore, we decided to study the influence of SO_2 on MND concentrations in red wines.

For this, we used a wine model solution spiked with MND (100 ng/L) to which increasing concentrations of SO_2 were added (0, 20, 30, 40, 60 mg/L). Table 6 shows that SO_2 had no effect on the level of MND in our experimental conditions. However, at high SO_2 levels there was a slight decrease (10%) in MND concentrations. Nevertheless, its reactivity is low compared to other monocarbonyls such as acetaldehyde, formaldehyde, or diketones such as diacetyl.[24] Therefore, we conclude that SO_2 at levels found in red wines is not able to bind MND and modify its concentration.

Incidence of the Type of Red Wine on the MND Formation during Bottle Aging. As aging in bottle is usually a very slow oxidative process even in the best conditions, the aging potential of a wine likely corresponds to its ability to resist a low, slow, and continuous oxidation. The notion of great wine (grand cru) is very important in enology. Besides their organoleptic attributes such as flavor complexity, low astringency, and well-balanced tannins, great wines are generally able to age for a long time, unlike more modest wines that develop their full potential after a relatively short period in the bottle.[25] This aging potential has also recently been echoed in the scientific literature in the "vin de garde" concept.[26,27] To illustrate this unique property of wines, we decided to compare the aging potential of two red wines from the same producer: a great (or first) wine and a second wine. The former is made to be better in terms of organoleptic complexity, structure, and aging potential. As stated by Langlois,[28] the "vin garde" concept is complex and involves a

Table 6. Effect of Sulfur Dioxide on Amount of MND Determined in Wine Model Solution after 7 Days ($n = 2$)

	SO_2					
	0 mg/L	10 mg/L	20 mg/L	30 mg/L	40 mg/L	60 mg/L
[MND] (ng/L)	100 (5)	100 (7)	98 (7)	99 (5)	97 (8)	85 (7)

wide variety of perceptual clues. However, both empirically and by using a sensory approach using overall evaluation (visual, olfactory, and mouthfeel) to assess wine, enologists are able to "predict" the ability of a young red wine to age or not. Great wines are known empirically to develop slowly, thus explaining the difference between great wines, which remain young for many years, and more modest wines, which rapidly take on a more oxidative nuance, as well as those that are saturated with oxygen during winemaking.

We quantified MND in two red wines corresponding to the first and second wines from the same producer and for five vintages (Figure 5). During the first years of aging, MND concentrations in both were under its perception threshold and were similar. From the 2003 vintage, however, there was a clear increase in the level of MND in the second wine. After 5 years of in-bottle aging, the MND concentration had risen to 73 ng/L in the first wine and to 120 ng/L in the second. Hence, by using a pertinent oxidation marker, we were able to validate a posteriori the result of wine-tasting sessions in which the attributes of the first and second wines from the same growth were established.

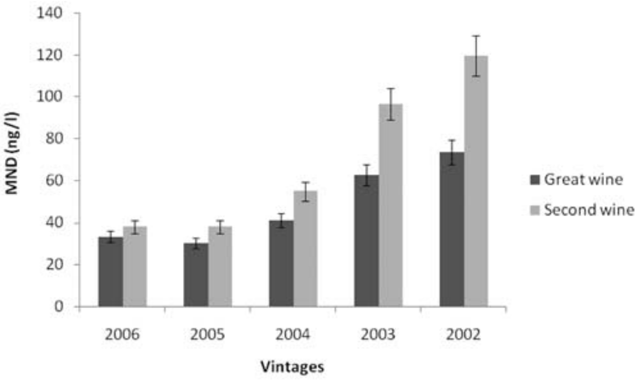

Figure 5. Comparison of MND concentrations found in great (first) and second wine from the same producer for several vintages. Analyses performed in duplicate in 2009.

We previously showed that MND formation in red wines is associated with an oxidative mechanism. Consequently, we cannot rule out that the higher MND concentrations in the second wine might result from a lower "antioxidant capacity", that is, from a sensory point of view, a lower ability to resist oxidative stress without any modification in the flavor of the wine. Oxygen transfer is a crucial parameter during bottle aging. Oxygen transfer is dependent on the oxygen dissolved at bottling and on the degree of permeability of the closure. For example, a recent finding[29] corroborated by other observations[30−32] reported that some synthetic closures can provide >6 mg/L of oxygen to wine in one year, whereas others provide <1 mg/L. However, apart from these data on the role of closures on wine aging in bottle, the reasons for these differences in aging potential are poorly understood and have not been studied from an enological point of view. Investigation of the precursors of MND and their degradation pathways would likely throw light on this issue.

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Funding

We thank the Bordeaux Wine Council (CIVB) and the Aquitaine Regional Government for funding this project.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Vincent Cruege (Vignobles André-Lurton) and Damien Bielle (Château Olivier) for kindly providing wine samples.

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