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# Natural oxygenation of Champagne wine during ageing on lees: A metabolomics picture of hormesis

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ABSTRACT

The oxygenation of Champagne wine after 4 and 6 years of aging on lees in bottle was investigated by FTICR-MS and UPLC–Q-TOF-MS. Three levels of permeability were considered for the stoppers, ranging from 0.2 to 1.8 mg/L/year of oxygen transfer rate. Our results confirmed a good repeatability of ultra-high resolution FTICR-MS, both in terms of *m/z* and coefficient of variation of peak intensities among biological replicates. Vintages appeared to be the most discriminated features, and metabolite annotations suggested that the oldest wines (2006) were characterized by a higher sensitivity towards oxygenation. Within each vintage, the oxygenation mechanisms appeared to be different for low and high ingresses of oxygen, in agreement with the hormesis character of wine oxygenation. In the particular case of single variety wines and for a given level of stopper permeability, our results also showed that variety discrimination could be easily achieved among wines.



## 1. Introduction

The role of oxygen in life has been extensively studied (Dowling & Simmons, 2009) and its impact on the winemaking process not only constitutes a remarkable example of hormesis (Arapitsas et al., 2012) but also addresses complex interplays, ranging throughout the elaboration steps from pure biological mechanisms to pure chemical ones. Numerous authors have already reported studies of these mechanisms (Karbowiak et al., 2009; Oliveira, Ferreira, De Freitas, & Silva, 2011). Many of these mechanisms were targeted to phenolic compounds considered to be primary substrates for oxidation, whether being enzymatic before the alcoholic fermentation, or chemical after (Danilewicz, 2011; Waterhouse & Laurie, 2006). In the case of dry white wine making, oxidation has become a major concern with the appearance of premature oxidation or untypical ageing. This is a phenomenon that can be seen in some recently bottled wines, which are supposed to age for years before reaching an organoleptic optimum, but actually exhibit colours and aromas of old wines (Schneider, 2014; Ugliano, 2013). To that respect, analyses were targeted in particular to the understanding of the origin of aroma descriptors; in particular, sotolon and aldehydes (Grant-Preece, Fang, Schmidtke, & Clark, 2013; Pons, Lavigne, Landais, Darriet, & Dubourdieu, 2010). However, given the symptoms associated with this untypical ageing, various authors have investigated the role of the permeability of bottle closures on the ageing potential of wine (Karbowiak, Mansfield, Barrera-García, & Chassagne, 2010; Ugliano et al., 2011). While low oxygen ingress through the closure does preserve the aromatic quality of a wine, when the oxygen inputs are too low, this can lead to non-desired reduced aromas (Ugliano et al., 2011). Non-targeted mass spectrometry-based metabolomics has recently shown great potential in describing the evolution of chemical spaces involved in enological practices (Arbulu, Sampedro, Gómez-Caballero, Goicolea, & Barrio, 2015; Gougeon et al., 2009; Jeandet et al., 2015; Liger-Belair et al., 2009). However, only few non-targeted studies have analyzed the oxygenation of wine so far, and they only concerned red wines (Arapitsas et al., 2012; Arapitsas, Speri, Angeli, Perenzoni, & Mattivi, 2014). Thousands of compounds have been

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identified in wine but only a very small fraction of these compounds are known to be antioxidant. Studies dealing with wine antioxidants were primarily focused on the behavior of the GSH grape-derived antioxidant, the exogenous $SO_2$ and ascorbic acid. Recent results report the absence of synergic antioxidant effect between ascorbic acid, glutathione and sulfur dioxide (Nikolantonaki, Magiatis, & Waterhouse, 2014). These results clearly demonstrated the lack of knowledge about the relative contribution of different species to the oxygenation-related chemistry and the existence of some hitherto unidentified antioxidants (Danilewicz, 2003; Danilewicz, 2007). Nevertheless, all these studies highlighted the extent of the yet unknown chemistry, which can be involved in enological processes, and the need for high resolution and sensitivity in mass measurements (Hertkorn, Harir, Koch, Michalke, & Schmitt-Kopplin, 2013; Roullier-Gall, Lucio, Noret, Schmitt-Kopplin, & Gougeon, 2014; Witting et al., 2014).

Like still wines, Champagne and sparkling wines are complex matrices made of compounds derived from all of the organisms (grapevine, yeast, bacteria, oak...) and processes involved in its production. However, the characteristic feature of Champagne compared to still wine is its super saturation with carbon dioxide ($CO_2$) dissolved molecules formed during the fermentation process, called *prise de mousse* (Liger-Belair, Polidori, & Jeandet, 2008). This $CO_2$ formation (together with ethanol) is the result of the addition of yeasts and sugar inside bottles already containing a still wine, called *base* wine, which are then kept sealed with caps and lying in a cellar for at least 15 months. Finally, after disgorging of these bottles to remove lees, a *liqueur d'expédition* may be added to adjust the desired sugar content before corking. When uncorked, these bottles release approximately 5 liters of dissolved $CO_2$ through the well-known and typical effervescence process (Liger-Belair, 2012). Three varieties of grapes are used in the production of Champagne: Chardonnay (white grape), Pinot Noir and Meunier (both red grapes), and depending on the desired organoleptic profile. Winemakers either make single variety Champagne or blend two or three of these varieties.

The key step in Champagne making is the second fermentation in bottles where a part of the $CO_2$ molecules produced by the yeast will be dissolved progressively into the Champagne, the other part will be concentrated under the cork, establishing equilibrium between the $CO_2$ in the two phases (Liger-Belair et al., 2008). Several studies of this step for the elaboration of sparkling wines have been reported, which highlighted the importance of the contact between wine and lees for the onset of enzymatic reactions. These reactions are responsible for the organoleptic trait of the final wine; either in terms of phenolic composition (Chamkha, Cathala, Cheynier, & Douillard, 2003) or in terms of volatile compounds, some of them being considered as age markers (Bosch-Fusté et al., 2007). However, Champagne wine making differs from sparkling wine making in that the aging on lees following the *prise de mousse* can last up to several years. During this fundamental step the initial *base* wine composition evolved first by biological and biochemical processes associated with the fermentation itself and finally by chemical processes (Valade et al., 2006). If the literature on Champagne has particularly focused on the physics and physical chemistry of the effervescence process (Liger-Belair, 2012; Liger-Belair et al., 2000; Liger-Belair, Vignes-Adler, Voisin, Robillard, & Jeandet, 2002) or the formation of adsorbed layers of amphiphilic macromolecules at the air/Champagne interface (Abou Saleh, Aguié-Béghin, Foulon, Valade, & Douillard, 2007; Peron, Meunier, Cagna, Valade, & Douillard, 2004); very few studies were precisely targeted on this several-year period of wine ageing. Valade et al. (2006) showed that as the produced $CO_2$ concentration increases, the yeast metabolism is rapidly decaying, leaving the $CO_2$-supersaturated wine composition under the prime influence of gas exchanges through closure. The huge difference in $CO_2$ partial pressure between the headspace (close to 6 bars at 12 °C) and the ambient air (around 0.0004 bar) forces $CO_2$ molecules to slowly escape outside of the bottle, while at the same time $O_2$ molecules invade the bottle (Liger-Belair, 2012; Valade et al., 2006). On the basis of repeated experiments over two years using synthetic seal-crown caps with controlled $CO_2$ permeability, these authors showed that upon ageing, there is a progressive increase of the oxygen diffusion into the wine, from the outside, following the laws of diffusion with a logarithmic phase (15–25 days depending on the cap) (Valade et al., 2006; Valade, Bunner, Tribaut-Sohier, Tusseau, & Moncomble, 2011; Vasserot, Jacopin, & Jeandet, 2001).

Here we report for the first time, a non-targeted metabolomics analysis of a series of Champagne wines after 4 and 6 years of ageing on lees. These bottles stopped with caps having three levels of controlled permeability to oxygen can be considered as a unique experimental setup for the identification of the chemical evolution of white wines upon slow oxygenation.

## 2. Material and methods

### 2.1. Champagne wines samples

A total of 27 bottles (75 cL) of Champagne wines from the same producer and from two vintages (2006 and 2008) were analyzed at the end of the ageing on lees, shortly after disgorging in July 2013. A series of samples from both vintages corresponded to blended *base* wines (⅓ Chardonnay, ⅓ Pinot noir, ⅓ Meunier) bottled for the *prise de mousse*, in July 2007 and July 2009, respectively. Thus, Champagne wines of this study were analyzed after 6 or 4 years of bottle ageing. For each vintage, bottles had been stopped for *the prise de mousse*, with synthetic seal-crown caps having three levels of controlled permeability to oxygen (Table 1). Thus for instance, bottles from the 2006 vintage, stopped with caps exhibiting permeability of 0.2, 0.7 and 1.8 mg/L/year of $O_2$, were analyzed after consumption of a total amount of 0.9, 3.15 and 8.1 mg of $O_2$, respectively. Sensory analyses of the corresponding wines repeated over several years have shown that low permeability consistently lead to wines with sulfur or reductive aromas, whereas high

**Table 1**
Characteristics for each sub-grouped samples.

| Sub-group | Variety | Vintage | Capsule permeabilitymg/L/years or $O_2$ | Number of samples (A–B–C) |
|---|---|---|---|---|
| PN. | Pinot Noir | 2008 | 0.7 | 3 |
| Meu. | Meunier | 2008 | 0.7 | 3 |
| Ch. | Chardonnay | 2008 | 0.7 | 3 |
| 07 Low | PN./Meu./Ch. | 2006 | 0.2 | 3 |
| 09 Low | PN./Meu./Ch. | 2008 | 0.2 | 3 |
| 07 Middle | PN./Meu./Ch. | 2006 | 0.7 | 3 |
| 09 Middle | PN./Meu./Ch. | 2008 | 0.7 | 3 |
| 07 High | PN./Meu./Ch. | 2006 | 1.8 | 3 |
| 09 High | PN./Meu./Ch. | 2008 | 1.8 | 3 |