**Role of closure and oxygen dissolved at bottling on white wine evolution:**

**a multiparametric approach**

Topo Angelo (topo.angelo@spes.uniud.it)

Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, 33100, Italy

Tutor: Prof. Comuzzo Piergiorgio

Co-tutor: Prof. Battistutta Franco

The aim of this PhD project is to study the evolution of a bottled white wine sealed with different closures, characterised by different oxygen transfer rates (OTR). In addition, a recent innovative technology for winemaking, processing with contactor membrane, was used to manage dissolved gases at bottling, in particular oxygen.

Ruolo della chiusura e dell’ossigeno disciolto all’imbottigliamento nell’evoluzione di vini bianchi: un approccio multiparametrico

Il presente progetto di dottorato si propone di studiare l'evoluzione di un vino bianco imbottigliato mediante l’utilizzo di differenti chiusure, caratterizzate principalmente da diversi *oxygen transfer rate* (OTR). Inoltre, è stata applicata una recente innovazione nel mondo enologico, la membrana *contactor*, per la gestione dei gas disciolti, in particolar modo dell’ossigeno disciolto.

**Key words**: Contactor membrane; closure; oxidation; aroma; carbonyl compounds; cyclic voltammetry.

# **1. Introduction**

The evolution of a bottled white wine is influenced by various parameters mainly related to the characteristics of the wine (Comuzzo *et al.* 2015; 2017; Kallithraka *et al.* 2009; Fracassetti *et al.* 2021), but especially by the packaging (bottle and closure) (Cantu *et al.* 2022; Lagorce-Tachon *et al.* 2016; Crouvisier-Urion *et al.* 2018) and storage conditions (Ferreira-Lima *et al.* 2013; Mas *et al.* 2002; Mafata *et al.* 2019; Wirth *et al.* 2012).

The International Organisation of vine (OIV) has authorised the use of contactor membranes (OIV-OENO 499-2013), which allow the management of dissolved gases (Schonenberger *et al.* 2019). This technique allows to reduce oxygen levels to concentrations below 0.2 mg/L (Schmidt *et al.* 2010). Contactors, potentially have a strong impact on evolution kinetics, while preserving the sensory and chemical characteristics of the wine.

In particular, the use of contactor membranes during the bottling phase would reduce the amount of dissolved oxygen, compensating the intense oxygenation related, for example, to filtration, which may contribute significantly to the amounts of oxygen dissolved at bottling (Day *et al.* 2015).

The management of oxygen in bottle is a critical factor, regulated by the performance of the closures (Silva *et al.* 2011; Skouroumounis *et al.* 2005; Godden *et al.* 2001). It is essential to carry out adequate maintenance of the bottling machine and, above all, to select a suitable closure with the appropriate oxygen transfer rate (OTR), for fulfilling a given winemaking project and producing wines with a good stability towards evolution.

Finally, in recent years, several research groups have studied new and faster analyses to determine the shelf-life of bottled white wine. In particular, cyclic voltammetry is a new technic in enology that can be useful in combination with different statistical approaches, to achieve this goal.

# **2. Materials and Methods**

## **2.1 Sample Preparation**

A Pinot Gris, vintage 2021, DOC delle Venezie, was selected for this research project. The wine was bottled at winery scale, using a GAI 3005 TOP integrated bottling machine (for technical cork closures), and a GAI 4292 corker (for screw caps) (GAI Macchine Imbottigliatrici Spa, Ceresole Alba, Italy). The wine was bottled in standard Bordeaux bottles, clear white, with a nominal volume of 750 mL at 20°C, with a BVS standard neck for the screwcaps.

Concerning oxygen management, two different treatments were applied to the wine: a control (no treatment) and a contactor membrane processing by using a Mastermind Remove equipment (Ju.Cla.S. S.r.l., Pescantina, Italy). Membrane had a nominal porosity of 0.05 µm and a MWCO < 50. The CO2 concentration after the treatments was 1.5 g/L and 0.9 g/L respectively and the O2 concentration was 1.2 mg/L in Control and 0.3 mg/L in membrane processed wine.

Four different closures were used for sealing the bottles: an agglomerated cork and three different screwcaps, one with a Saranex® liner, and two alternative liners named “M” and “Z”, characterized by a lower OTR. Table 1 summarizes all the trials. Samples were stored at 20°C ±5°C and 70% ±10% relative humidity until analysis, carried out after three and eleven months.

**Table 1** *Codes used for the different closures and estimated oxygen transfer rate.*

|  |  |  |
| --- | --- | --- |
| Sample code | Closure | Theoretical OTR |
| SUG | Cork |  *n.k.1* |
| SAR | Saranex® | +++ |
| LIN\_M | Liner "M" | ++ |
| LIN\_Z | Liner "Z" | + |

*1 n.k.: not known*

**2.2 Oxygen dissolved**

The oxygen dissolved in bottles was measured as described by Comuzzo et al. (2017).

**2.3 Determination of Volatile Compounds**

Volatile compounds were analysed by HS-SPME-GC-MS, using GC2030 Nexis gas chromatograph (Shimadzu, Kyoto, Japan) coupled with a QP2020NX mass spectrometer and an HT2800T autosampler (HTA S.r.l., Brescia, Italy). A divinylbenzene/carboxen/polydimethylsiloxane (DVB-CAR-PDMS, 50/30 μm x 2 cm) fiber (Supelco, Bellefonte, PA, USA) was used for SPME. Column was a DB-Wax 30 m x 0.25 mm i.d. x 0.25 μm (Agilent Technologies, Santa Clara, CA, USA) and the carrier gas was helium at a flow rate of 35 cm/s.

Ten millilitres of samples were added to 3 g of NaCl in 20 mL glass vials and immediately sealed. Ethyl heptanoate (Sigma-Aldrich, St. Louis, MO, USA) was used as internal standard at 2.17 mg/L. The analysis was carried out setting the following conditions: incubation for 15 minutes at 40 °C, under stirring (500 rpm 5 seconds on and 2 seconds off), microextraction for 15 minutes, desorption for 5 minutes of the fiber into the injector, in splitless mode. The initial oven temperature was 40 °C held for 5 minutes; then it was ramped at 4 °C/min up to 240 °C, held for 15 minutes. For the qualitative analysis, mass spectra were acquired at 70 eV and compared with those reported in the NIST 20 mass spectra library. Moreover, linear retention indices were calculated, using *n*-alkanes (C7-C30) (Sigma-Aldrich, St. Louis, MO, USA), and compared with those found in literature. Finally, for some of the detected compounds identification was confirmed by comparison with commercial standards. Semi-quantitative analysis was carried out by the internal standard method.

For carbonyl compounds, the method proposed by Moreira *et al.* (2019), based on *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) derivatisation, was used with some modifications. Internal standard was *p*-fluorobenzaldehyde at 0.64 mg/L in wine (Sigma-Aldrich, St. Louis, MO, USA). Chromatographic conditions were as above, while microextraction was held for 45 min.

**Table 2** *Composition of the chemical classes in which volatile compounds were grouped.*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Ethyl esters | Acetic esters | Other esters | Ageing markers | Alcohols | Acids |
| Ethyl butanoate   | 3-methyl-1-butanol acetate | Isopentyl hexanoate | Diethyl succinate | 2-methyl-1-Propanol | 2-methyl-propanoic acid |
| Ethyl hexanoate | Hexyl acetate | Propyl octanoate | Ethyl 9-decenoate | 3-methyl-1-butanol | Butanoic acid |
| Ethyl octanoate | 2-phenylethyl acetate | Caprylic acid, isobutyl ester |   | 1-Hexanol | Hexanoic acid |
| Ethyl decanoate |   | Octanoic acid, 3-methylbutyl ester |   | 1-Octanol | Octanoic acid |
| Ethyl dodecanoate |   |   |   | 1-Decanol | Decanoic acid |
| Ethyl tetradecanoate |   |   |   | 2-phenylethanol |   |
| Ethyl hexadecanoate |   |   |   |   |   |

**2.4 Cyclic voltammetry**

Cyclic voltammetry was performed by the setup proposed by Comuzzo et al. (2017), with a modification. For the electrochemical analysis, screen-printed Metrohm 110 was used (Metrohm Italiana Srl, Origgio, Italy).

**2.5 Statistical Analysis**

Statistical analysis was performed with software Statistica 8 (Statsoft Inc., Tulsa, OK, USA) to obtain statistical significance using a two-way ANOVA followed by Tukey's post-hoc test; R 4.2.1 (R Development Core Team) to perform the Principal Component Analysis (PCA).

# **3. Results and Discussion**

**3.1 Dissolved oxygen**

Dissolved oxygen was measured three days after bottling in order to allow the equilibration of the wines and repeated every two months.

The first measurement showed a significant difference between membrane processed and control samples, except for the wines sealed with technical cork (Figure 1). In the following 95 days, the samples closed with screwcaps showed a slower oxygen consumption with respect to those closed with cork. From day 144 to 424, oxygen concentration reached a stability and screwcap had lower levels with respect to cork closures, with similar behavior, probably due to their lower OTR.

a

**Figure 1**  *Kinetic of oxygen consumption after bottling, in (a) control samples and (b) samples treated by contactor membrane.*

**3.2 Aroma Compounds (ACs)**

The characterisation of the volatile composition (Table 3) registered significant differences only for ethyl esters at the first sampling point, after three months of storage. This difference is mainly related to ethyl octanoate (Table 4); this ester was which is the most abundant among ethyl esters and its concentration was higher in the samples treated with the contactor membrane. The same trend was observed for the other aroma groups (Table 3), even if without significant differences.

After eleven months of bottle storage, statistical differences among samples increased, particularly for ethyl esters, ageing compounds and organic acids, with a generalized lower amount in the membrane-treated samples.

Between the two different sampling points, there was a strong decrease in acetic esters, organic acids and other esters. Instead, there was a decrease of ageing compounds after membrane processing, except for Saranex. Table 4 summarizes the molecules that changed the most over time, between the two sampling points.

**Table 3** *Volatile composition of the samples after 3 and 11 months (ACs divided in groups as in Table 2; values in µg/L). Different letters indicate significant differences between samples of the same sampling point to ANOVA e Tukey HSD test (p <0,05).*



**Table 4** *ACs with the greatest variation between 3 and 11 months (ACs divided in groups as in Table 2; values in µg/L). Different letters indicate significant differences between samples of the same sampling point to ANOVA e Tukey HSD test (p <0,05).*



**3.3 Cyclic voltammetry**

The values of current intensity (µA) of the anodic trace of the cyclic voltammograms were analysed by PCA. At the first sampling point, samples showed the tendency to have a slightly higher curve with the application of the contactor membrane (Figure 2). This could be related to the lower level of dissolved oxygen at bottling, and thus to the greater preservation of the phenolic fraction.

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**Figure 2**  *Principal Component Analysis (PCA) on anodic trace data of cyclic voltammetry, after three months of bottle storage. For each sample, data from triplicate analysis were used.*

**3.4 Carbonyl compounds**

The method for the quantification of carbonyl compounds, developed by Moreira et al. (2019) for Porto wines, demonstrated to be a useful tool for the analysis of wine evolution.

The carbonyl compounds detected in this work are: hexanal (herbaceous), 2- and 3-methylbutanal (fruity odor), propanone (solvent) and phenylacetaldehyde (honey).

In Figure 3, it can be seen that liner "M" and Saranex, treated with a contactor membrane to manage gases, contain a lower amount of carbonyl compounds. This effect could be related to a lower concentration of oxygen during the storage period. This behaviour does not appear for the liner "Z", and this could be related to a greater rigidity of the liner; if this hypothesis will be confirmed in future experiments, the sealing properties of this liner could be improved by a higher pressure applied at corking.

 

**Figure 3**  *Principal Component Analysis (PCA) based on absolute areas detected for carbonyl compounds, after 11 months of bottle storage. For each sample, data from triplicate analysis were used.*

# **4. Conclusions and Future Perspectives**

Results demonstrated that the use of contactor membrane could help to reduce the initial concentration of dissolved gases, especially oxygen, allowing a better evolution of the wine over time. This technique, combined with an appropriate closure selection, can improve the evolution of white wines.

Further analysis will be carried out on the data collected in terms of chemical composition, electrochemical and sensory data; moreover, additional samplings will be performed to better understand the mechanisms involved in the evolution of the different samples.

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