“SHORT TERM EFFECTS OF PRE-FERMENTATIVE HYPEROXYGENATION ON CHARDONNAY WHITE WINES”

“IN RELATION TO SENSORY CHARACTERISTICS, COLOR-RELATED PHENOLICS AND CULTIVAR TYPICALITY”

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ABSTRACT

Hyperoxygenation is a pre-fermentative technique used to eliminate the major fraction of phenols, the main substrate of oxidation, to reduce the risk of future colour browning. The current study was aimed to assess the effect, in a short term evaluation period, of must hyperoxygenation on a non-aromatic cultivar such as Chardonnay, in order to further research into a topic not deeply investigated so far. Attention was focused not only on the total phenolic content and the effects on colour, but also on the aromas, their stability and especially the perceived typicality of the grape variety considered.

Two batches of Chardonnay, harvested in SA, were submitted to conventional vinification and vinification with the addition of the hyperoxygenation technique. Oxygen was added throught manual aeration until the must's color reached an intense brown tone. Juices and wines were evaluated chemically and sensorially. Finally, the results were statistically analysed.

Juices and wines showed differences according to the treatment. Phenolic compounds were successfully removed and phenolic acids were the most affected by the treatment. Browning susceptibility was found to be reduced in the treated wines of both batches. Chardonnay typicality was not lost due to the treatment while the aromatic profile showed slight differences between both batches and treatments. Must composition and the level of grape ripeness played an important role in the evolution of the parameters chemically analysed and on the sensory perception.

Hyperoxygenation proved to be a successful technique in reducing colour browning susceptibility and aroma predisposition towards oxidation-related aromas while maintaining the cultivar typicality and reducing the use of sulphur dioxide during vinification. It could be a valid technique also in case of press juices, white musts with high phenolic content and base wine for sparkling wine production.

Keywords: hyperoxygenation, Chardonnay, phenolics, browning, typicality.

RESUMO

A hiperoxigenação é uma técnica pré-fermentativa usada para eliminar a maior parte dos compostos fenólicos, principais substratos da oxidação, reduzindo o risco futuro de acastanhamento da cor. O presente estudo teve como objetivo avaliar o efeito da hiperoxigenação do mosto em uma casta não aromática como a Chardonnay. A atenção foi focada não apenas no conteúdo fenólico total e nos efeitos sobre a cor, mas também nos aromas, na estabilidade e, principalmente, na percepção da tipicidade da casta considerada.

Dois lotes de Chardonnay, colhidos na África do Sul, foram submetidos à vinificação convencional e a

vinificação com a execução da técnica de hiperoxigenação. O oxigênio foi adicionado através da aeração manual até que a cor do mosto atingisse um tom marrom intenso. Mostos e vinhos foram avaliados quimicamente e sensorialmente. Finalmente, os resultados foram analisados estatisticamente.

Mostos e vinhos apresentaram diferenças de acordo com o tratamento. Os compostos fenólicos foram removidos com sucesso e os ácidos fenólicos foram os mais afetados pelo tratamento. Verificou-se que a suscetibilidade ao acastanhamento da cor foi reduzida nos vinhos tratados de ambos os lotes. A tipicidade de Chardonnay não foi perdida devido ao tratamento, enquanto o perfil aromático mostrou pequenas diferenças entre os lotes e os tratamentos. A composição do mosto e o nível de maturação da uva desempenharam papel importante na evolução dos parâmetros quimicamente analisados e na percepção sensorial.

A hiperoxigenação provou ser uma técnica bem-sucedida na redução da suscetibilidade ao acastanhamento da cor e a predisposição de aromas relacionados à oxidação, mantendo a tipicidade da cultivar e reduzindo o uso de dióxido de enxofre durante a vinificação. Pode ser uma técnica válida também no caso de mostos de prensa, mostos brancos com alto teor fenólico e vinho base para produção de espumante.

Palavras-chave: hiperoxigenação, Chardonnay, compostos fenólicos, acastanhamento, tipicidade.

RESUMO ALARGADO

A hiperoxigenação é uma técnica pré-fermentativa usada para eliminar a maior parte dos compostos fenólicos, principais substratos da oxidação, reduzindo o risco futuro de acastanhamento da cor. O presente estudo teve como objetivo avaliar o efeito da hiperoxigenação do mosto em uma casta não aromática como a Chardonnay. A atenção foi focada não apenas no conteúdo fenólico total e nos efeitos sobre a cor, mas também nos aromas, na estabilidade e, principalmente, na percepção da tipicidade da casta considerada.

O ensaio foi realizado em dois lotes diferentes de uvas Chardonnay, provenientes de dois vinhas separados na área de Stellenbosch (África do Sul). Os dois lotes foram submetidos à vinificação convencional e vinificação com execução da técnica de hiperoxigenação. O oxigênio foi adicionado através da aeração manual até que a cor do mosto atingisse um tom marrom intenso. Foram realizadas análises químicas e sensoriais, seguidas de análises estatísticas dos resultados. As principais análises químicas realizadas foram: análise UPLC-MS/MS sobre glutationa reduzida e oxidada (ppm); Análise não segmentada por HR-MS, análise por HPLC sobre etanol (g/L e% v/v), glicerol (g/L), principais açúcares (g/L) (sacarose, glicose e frutose), ácidos orgânicos (g/L) (ácidos tartárico, málico, cítrico,

succínico, lático e acético). Além disso, medições colorimétricas foram realizadas através de um espectrofotômetro e os parâmetros determinados foram: fenóis totais (A280), ácidos hidroxicinâmicos (A320), flavonóides (A360) e pigmento amarelo, indicador de acastanhamento (A420). A análise do CieLab também foi realizada para acompanhar a cor dos mostos e vinhos.

A análise sensorial foi realizada por um painel de 20 especialistas e a avaliação foi concluída em uma sessão. Foi feita uma única sessão de prova com as 12 amostras contendo os dois lotes de vinhos da casta Chardonnay. Em cada lote foram avaliados 3 repetições para os vinhos de controle e 3 repetições para os vinhos hiperoxigenados. Todas as amostras foram apresentadas em ordem aleatória. Foi solicitado aos juízes que avaliassem quanto os vinhos eram representativos para a cultivar Chardonnay. Além disso, o perfil de aroma dos vinhos foi descrito através de um método rápido, o CATA ("Check-all-that-apply"). Foi solicitado ao painel que selecionasse, a partir de uma lista predeterminada de atributos, o maior número possível de termos que caracterizassem o aroma de cada amostra. A análise de correspondência (CA) foi realizada com dados sensoriais da avaliação do CATA utilizando a frequência de citação.

A técnica de hiperoxigenação produziu mudanças significativas nos mostos e nos vinhos. Foram detetadas diferenças significativas em diversos parâmetros analisados. No mosto, a glutationa na sua forma reduzida estava significativamente em maior concentração nas modalidades não hiperoxigenadas comparativamente às hiperoxigenadas. A ausência de dióxido de enxofre e oxigênio fornecido causou um aumento na ligação da glutationa com as o-quinonas para formar GRP (Grape Reaction Product). A glutationa exerceu sua atividade antioxidante até sua depleção, que foi mais rápida nos mostos tratados.

Os compostos fenólicos foram removidos com sucesso com hiperoxigenação e os ácidos fenólicos foram os mais afetados pelo tratamento. Sensorialmente falando, os ácidos fenólicos não afetam notavelmente as propriedades organolépticas de um vinho. Esta é provavelmente a razão pela qual não foram encontradas grandes diferenças em termos de amargura, adstringência e cor.

Os resultados mostraram que, em geral, quanto maior a concentração fenólica inicial, maior o efeito do tratamento com hiperoxigenação. Além disso, um lote mais maduro (lote 2), com menor conteúdo inicial de GSH, maior teor de polifenóis e maior pH, apresentou maior perda de polifenóis, mesmo nos vinhos controle. A perda de flavonóides foi estatisticamente significante apenas entre as etapas da vinificação, independentemente do tratamento. Apesar disso, a diminuição ocorrida nos flavonóides, especialmente no lote 2, ainda pode ser praticamente relevante no caso de mosto de prensa que contenham quantidades superiores deste composto fenólico. Verificou-se que a suscetibilidade ao acastanhamento da cor foi reduzida nos vinhos tratados de ambos os lotes. Além disso, foram encontradas diferenças significativas no acastanhamento da cor de acordo com o estágio da vinificação. Os dados mostram quão escura é a

cor dos mostos tratados, em comparação com os controles, nos primeiros estágios da vinificação. No entanto, a luminosidade volta após clarificação e fermentação alcoólica.

A tipicidade de Chardonnay não foi perdida devido ao tratamento, enquanto o perfil aromático mostrou pequenas diferenças entre os lotes e os tratamentos. O segundo lote, com um estado de maturação mais alto, é caracterizado por tipos de aromas 'derivados da oxidação' mais frequentes em ambos os grupos de vinhos (tratados e não tratados). Além disso, a nota 'láctico/amanteigado' e 'marmelada' foram percebidos com mais frequência em comparação com o primeiro lote. O primeiro lote, por outro lado, permaneceu em geral mais cítrico, frutado e floral (notas de 'flor de laranjeira' em particular). As diferenças entre lotes devem-se às diferentes composições de mosto e estão de acordo com o estado de maturação das uvas. Os vinhos tratados foram, em geral, mais frequentemente caracterizados por aromas de 'cítricos', notas de 'pão torrado', 'figos secos', 'caramelo' e 'mineralidade'. Além disso, as notas de 'frutas amarelas' e 'frutas tropicais' são comuns aos vinhos de controle. Os vinhos de controle foram mais frequentemente associados a aromas florais ('flor de laranjeira'), frutas amarelas e tropicais ('banana' e 'abacaxi'). Além disso, os aromas 'relacionados à maçã' foram encontrados com mais frequência nos grupos controle, especialmente as notas de 'maçã oxidada' no segundo lote. Isso demonstra a maior sensibilidade ao oxigênio dos vinhos não tratados em termos de perfil aromático, o que significa que após a hiperoxigenação, um risco menor de obter aromas relacionados à oxidação pode ser alcançado.

A composição do mosto e o nível de maturação da uva desempenharam papel importante na evolução dos parâmetros quimicamente analisados e na percepção sensorial. Verificou-se que um maior amadurecimento é um possível responsável pelo aumento dos aromas relacionados à oxidação, mas em menor grau quando se trata de vinhos hiperoxigenados. Portanto, essa técnica pode ser uma boa solução no caso de uvas maduras demais, para reduzir o risco de desenvolver aromas indesejados relacionados à oxidação. Mais estudos devem ser realizados para avaliar os efeitos da hiperoxigenação na estabilidade da cor, principalmente após um período mais longo de armazenamento. Uma análise sensorial também deve ser incluída para avaliar se eventuais diferenças visuais na cor podem ser detectadas ou não pelo painel.

A hiperoxigenação provou ser uma técnica bem-sucedida na redução da suscetibilidade ao acastanhamento da cor e a predisposição aos aromas relacionados à oxidação, mantendo a tipicidade da cultivar e reduzindo o uso de dióxido de enxofre durante a vinificação. Pode ser uma técnica válida também no caso de mostos de prensa, mostos brancos com alto teor fenólico e vinho base para produção de espumante.

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1 INTRODUCTION

The aim of this project is to assess the effect of must hyperoxygenation on a non-aromatic cultivar such as Chardonnay, within a short term evaluation period. This pre-fermentative treatment is currently used by some producers in order to eliminate the major fraction of phenols, the main substrate of oxidation, with the aim of reducing the risk of future color browning. Another aspect is the reduction in the use of sulfure dioxide to prevent oxidation in the first stages of vinification. The open question concerns how much this type of winemaking technique might affect not only the total phenolic content and the effects on the color but also the aromas, their stability and especially the perceived typicality of the wine.

The objectives of this work consist of a chemical evaluation of the control juices and wines in comparison to the ones subjected to hyperoxygenation. Furthermore, a sensory evaluation was performed to compare the treated and the untreated wines. The experiment was carried out on two different Chardonnay grape batches, coming from two separate vineyards within the Stellenbosch area (South Africa). Grapes were divided and subjected to the treatments above mentioned.

Hyperoxygenation is a technique not deeply investigated so far. Most of the work or experiments found in the literature are dated back to the 1990s, with a few recent exceptions. This is why further research into the topic was conducted, with the main focus on the effects that this pre-fermentative treatment has on the wine's sensory characteristics, quality and typicality of young Chardonnay wines.

2 LITERATURE REVIEW

2.1 The concept of hyperoxygenation

To prevent or reduce the browning effect, caused by phenolic oxidation, there are different approaches to consider and different strategies that can be applied before fermentation. More traditional ways are based either on a lower phenolic extraction, throught gentle pressing and short contact with the skins, or on the protection of the phenolics extracted. Grapes and musts in fact, can be processed under inert conditions through the use of carbon dioxide or nitrogen, avoiding contact with oxygen and therefore reducing the concentration of the oxidated polymers *(Mayèn et al. 1996).* Finally, sludge removal can also help to reduce the substrate more susceptible to oxidation *(Mayèn et al. 1996*). However, extracting less phenolics or protect the ones extracted are not the only possibilities. A third option would be to remove the phenolics alrady extracted and this could be done throught hyperoxygenation.

"Hyperoxygenation is a pre-fermentative technique characterized by an external oxygen addition to a non-sulfited must until saturation" (*Cejudo-Bastante et al., 2011).* The purpose is to favor the enzymatic oxidation of certain phenolic compounds, sensitive to the action of oxygen, allowing their transformation into oxidized polymers of high molecular weight, characterized by the brown color. After clarification, the polymers precipitated will be removed in order to reduce not only the risk of browning during wine storage but also bitterness and astringency, caused by the phenolic compounds that will be present in musts and wine in much lower concentrations. On the other hand, if phenolic compounds are oxidized in the wine they may cause an alteration in flavor and color during aging (*Cejudo-Bastante et al., 2011).*

As stated by *Schneider (1998),* oxidation on purpose before alcoholic fermentation can improve wine's shelf-life. The removal of the principal substrates, in fact, makes wines coming from oxidized must much more stable against oxygen during aging. One of the the traditional approaches during winemaking is focused on lowering the effect of the Polyphenoloxidase (PPO) activity. It can be done through the use of high temperatures (pasteurizing the wine to cause the inhibition of tyrosinase activity), adding sulfur dioxide with the same inhibition purpose, or performing bentonite fining which eliminates part of the soluble enzyme *(Zironi et al., 2010)*. In the case of hyperoxygenated wines, these procedures should be avoided, otherwise, the elimination or the heavy reduction of tyrosinase activity will definitely limit the flavonoids removal, which is the main purpose of this technique.

A further positive effect coming from hyperoxygenation would be a reduction in the use of sulfur dioxide, generally used to prevent browning at the must stage and during bottle storage. Sulfur dioxide

can be added to the must to prevent microbiological spoilage but after the treatment and subsequent clarification, or later on in the wine to avoid the malolactic fermentation, as suggested by *Cejudo-Bastante et al. (2011).*

Clarification, or even centrifugation, is an important step to remove the brown suspended solids after phenolic precipitation. If the juice is not clarified properly, the precipitates can be dissolved again in the presence of alcohol or sulfur dioxide, during fermentation *(Schneider, 1998).* Concerning the color after the treatment, it will acquire heavy brown notes but after sedimentation and clarification, it will turn bright again *(Cheynier et al., 1991)*. Enzimatically induced polymerization in musts produces brown pigments which are insoluble within the must medium and therefore easily removable. In case of filtration prior to fermentation, the brightness will be reached immediately, demonstrating how the responsible compounds for the brown notes are the physically removed precipitated solids *(Schneider, 1998).*

Finally, pre-fermentative hyperoxygenation is not influencing fermentation kinetics because all the oxygen is consumed enzymatically in a short period of time and it will not be available for the yeasts during the exponential fermentation phase *(Schneider, 1998*). The possible ways concerning how to perform this tecnique are explained in detail in subsection 2.4.

2.2 The effects of oxygen in wine compounds

2.2.1 The role of oxygen in winemaking

Oxygen can bring both positive and negative effects on musts and wines. On the negative side, an excess of oxygen can lead to color browning and to a loss of color due to the phenolic oxidation and to a decrease of the varietal aromas, together with the appearance of new oxidative off-flavors *(Ribéreau-Gayon et al., 2006)*. On the other hand, the positive sides concern the stabilization of the phenolic fraction and the consequent reduction of the perceived bitterness and astringency, as well as a contribution to the aroma evolution during aging *(Ribéreau-Gayon et al., 2006).*

According to *Zironi et al. (2010),* the reasons behind the balance between the cases above mentioned, are strictly dependant on:

i. The amount of oxygen dissolved: a higher dissolution gives better chances for oxidative reactions to happen.

ii. The winemaking stage: due to the type of oxidative reaction. In musts, it is enzymatically catalyzed by PPO. The reactions are faster and higher is the browning effect, compared to the chemical oxidation taking place in the wines.

iii. The juice and wine composition: specifically the concentration of reducing agents or antioxidant compounds in it. They can be either found in the grapes (glutathione, polyphenols) and are strictly dependant on the grape variety considered, or they can be exogenous substances that can be added (sulfur dioxide and ascorbic acid).

iv. Temperature also plays a role in the dissolution rate of O_2. At higher temperatures (20-25°C) the saturation level is reached at 6-7 mg/L, whilst at around 5°C it increases up to 10 mg/L. On the other hand, the oxidation reactions are faster at 30°C rather than 20°C.

v. Time of exposure increases the effects of oxidative reactions, especially if the wine composition lacks in antioxidant compounds.

Oxygen consumption in musts by the PPO system is generally a fast process, which is measured in mg/L of flavonoids to mg/L oxygen *(Schneider, 1998).* The main factors related to this process are the initial hydroxycinnamic acids content in the must and the molar ratio between hydroxycinnamic acids and glutathione, which affects the oxidation kinetics the most. Winemaking techniques such as maceration or using high pressures when extracting the juice, can, of course, increase the flavanol extraction and lead to higher oxygen consumption *(Cheynier et al., 1991).* The level is higher in the absence of sulfur dioxide, considering that the addition of SO_2 inhibits tyrosinase activity and consequently reduces the catalyzing effect of oxygen during the enzymatic reaction.

It was proved by *Schneider, (1998)* that oxygen consumption in the must generally ranges from 30 to 200 mg/L/hr and half of the oxygen is consumed within the first hour of contact. Once the phenolic substrate depletes, the oxygen uptake it's drastically reduced. An experiment was conducted by the author in order to monitor the oxygen demand at atmospheric pressure during 180 minutes for 20 different cultivars and the results showed how phenols stop precipitating only 15 minutes after having reached the saturation level. The same behavior was shown by most of the cultivars. Under industrial conditions, the maximum time for flavonoids precipitation took 30 to 60 minutes, with a continuous oxygen supply for two hours. An average level of 50 mg/L of oxygen was absorbed between all cultivars and 95% of the values ranged from 30 to 65 mg/L of absorbed oxygen. In the cases of incomplete precipitation, the residual content was enough to be absorbed by yeasts during fermentation. As a general rule, as oxygen consumption continues, the flavonoid precipitation rate decreases. The contact with the solid parts, the higher extraction of phenols and the more mechanical treatments, cause an increase of the phenolic substrate and the capacity the juice has to consume oxygen.

The balance between the oxidation-reduction reactions is reached thanks to the activity of oxidative agents and reducing ones. Other compounds though can act as powerful catalyzers. This is the case of

metals like copper and iron which mediate the reaction of polyphenols with oxygen and accelerate the oxidative processes in wine. Nonmetal catalized pathways, in fact, unlikely play a significant role in these processes *(Danilewicz, 2007).* Copper and iron generate peroxides and free radicals which can further oxidize other compounds and lead to the formation of acetaldehyde (MeCHO) from ethanol oxidation. Acetaldehyde can induce the production of acetals, other aroma compounds responsible for the typically oxidized off-flavor, as reported by *Zironi et al. (2010).*

Sulfite accelerate the rate of oxygen uptake and the reaction of polyphenols with oxygen. It then reduces the produced quinones back to polyphenol, thus preventing their depletion. Therefore, sulfite plays its antioxidant role by both polyphenol regeneration and hydrogen peroxide interception, preventing in this last case etanol oxidation *(Danilewicz, 2012).*

2.2.2 Differences in oxidation mechanisms between musts and wines

The molecules responsible for the oxidative browning of wines are considered to be the *o*-diphenols. In musts is the enzymic browning that mostly changes the color intensity and tone, whereas in wines it is mainly the non-enzymic one. Tyrosinase and laccase are polyphenoloxidases (PPOs), enzymes naturally found on grapes and responsible for the phenols transformation into o-quinones and the consequent browning reactions in musts *(Du Toit et al., 2006).* Oxygen behaves as a catalyzer for those enzymes and this is why it is generally avoided during the first stages of vinification. While tyrosinase is always present on grapes, laccase is present mainly on moldy grapes and it has a much broader spectrum of action and less sensitivity to powerful antioxidant such as sulfur dioxide. Tyrosinase is quite sensitive to SO_2 and it can be easily removed by bentonite treatments whereas laccase is poorly affected by both *(https://www.awri.com.au/wp-content/uploads/managing_botrytis_infected_fruit_fact_sheet.pdf)*

Focusing on healthy grapes, tyrosinase has its strongest activity on caftaric acid, as confirmed by *Zironi et al. (2010)* and *Oszmianski et al. (1996).* Caftaric acid is a monophenol and a derivate of the hydroxycinnamic acids. It is one of the most present phenolic compounds found in white grapes and it is easily converted into an o-quinone by tyrosinase, as shown in Fig. 2-1. Once the *o*-quinones are present, they can further react according to their redox properties and electronic affinities, successively causing a fast polymerization into other brown pigments, especially when the pH value is high *(Li et al., 2008).*

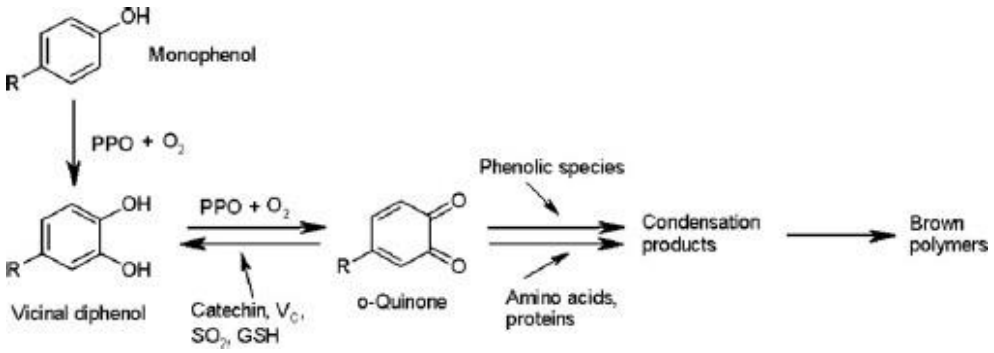


Figure 2-1. Musts browning process caused by PPO and oxygen (Li et al., 2008).

The initial hydroxycinnamic acid content and the presence of oxygen are the main responsible for the browning degree on white musts *(Schneider, 1998).* The consumption rate of oxygen by this enzymatic reaction, is reliant on the content of the abovementioned compounds, while the kinetics of oxidation is mainly related to the molar ratio between the hydroxycinnamic acids and glutathione content, dependent in turn by the grape variety *(Cheynier et al., 1990).* The ratio value gives a good representation of the must oxidation susceptibility. The higher the value, the darker the musts. According to *Kritzinger et al. (2012*), values of 0,9-2,2 represent a lightly colored must while a medium colored one would be between 1,1-3,6 and finally 3,8-5,9 characterize a darker must.

Glutathione is a tripeptide made by glutamate, cysteine, and glycine and it plays an important role in protecting phenolic compounds from browning. It combines with caftaric acid *o*-quinones which are generated by the enzymic oxidation of caftaric acid, the major phenol of white musts. The result is a colorless compound named 2-S-glutathionyl caftaric acid or Grape Reaction Product (GRP) *Kritzinger et al. (2012)*. Enough glutathione can, therefore, trap the o-quinones and reduce the formation of browning pigments. If the content of copper in the must is high though, it can compete with the o-quinones and react with the glutathione, decreasing its content and antioxidant activity *(Li et al., 2008).* Once glutathione is depleted, the excess of o-quinones can easily react and oxidize other compounds such as flavanols (catechins and procyanidins) and the GRP itself. Both can be reduced back to caftaric acid which is reformed again. Quinones from caftaric acids can also polymerize with their own precursors and a further re-oxidation of the compounds will be possible. In the case of laccase activity, coming from moldy grapes infected by *Botrytis Cinerea*, even the GRP itself can be a substrate for the enzymatic oxidation *(Kritzinger et al., 2012).*

Contrarily, chemical oxidation occurs mainly in the wine medium and it can be catalyzed by the presence of light and especially by copper and iron. As shown in Fig. 2, phenols can get oxidized and consequently polymerize forming other oxidized products. *o*-Diphenols can oxidize into *o*-quinones and oxygen is

reduced to H_2O_2, which in presence of ferrous ion (Fe^{2+}), might originate very reactive species like hydroxyl radical (HO•), a powerful oxidant agent. The higher the production of H_2O_2, the higher is the production of acetaldehyde in association with transition metals, by etanol oxidation. As a consequence, phenols which were not sensitive to the oxygen action become prone to oxidation. Tartaric acid and ethanol, being two of the compounds present in the highest concentration in wine, are indeed avaliable substrates for this reaction. Through the oxidation by HO•, they form glyoxylic acid and acetaldehyde respectively. The oxidation of phenols through the non-enzymatic way can be very fast when the pH is high but is slower within an acidic medium like wine *(Li et al., 2008).*

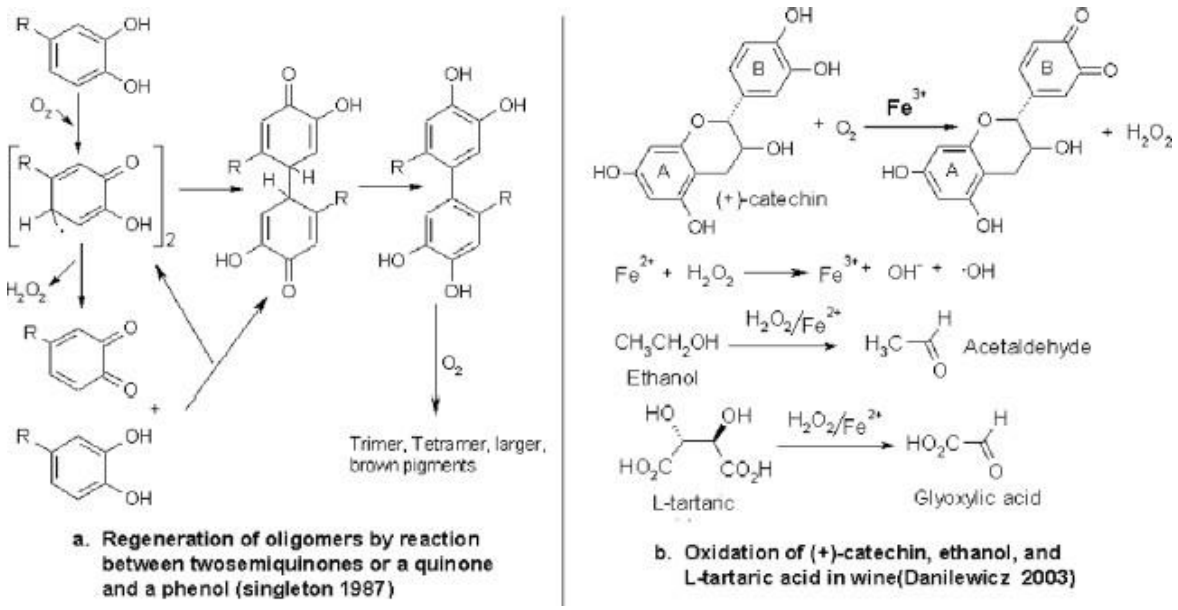


Figure 2-2. Different type of chemical oxidation reactions taking place in wine (Li et al., 2008).

Summing up the differences, oxidation in musts is an enzymatic process, whereas when it comes to wines, the type of catalysis become chemical and not enzymatic anymore. In addition to that, the rate of *o*-quinones production in the must is much faster than in the wine, due to the presence of the PPO. Finally, the brown pigments resulting from oxidative reactions are insoluble in must but partially soluble within an alcoholic medium. This is why it is easier to deplete those compounds before fermentation and this is why hyperoxygenation is a technique applicable at the juice stage. Apart from causing a depletion of the substrates most sensitive to oxidation, such as phenolic compounds, it removes until a certain extent the tyrosinase as well. When phenolic compounds have been oxidized in the wine instead, they cause an alteration in flavor and color during aging *(Li et al., 2008).*

2.3 Hyperoxygenation effects

2.3.1 Hyperoxygenation effects on color-related phenolics

As previously mentioned, the purpose of hyperoxygenation is the removal of part of the phenolic compounds in grape must, relying on the natural process of enzymatic oxidation in order to reach a better color and sensory stability. Finished white wines will, therefore, contain a lower amount of polyphenols and a higher content of Grape Reaction Product compared to non-hyperoxidized wines characterized by both high polyphenol content and browning potential *(Li et al., 2008).*

Within the phenolic compounds affected by this reaction, the flavonoids are the main compounds influencing the organoleptic characteristics of the wine, making the difference in terms of decreasing bitterness and astringency since these monomeric precursors of tannins will be largely removed *(Schneider, 1998).* Furthermore, their thresholds decrease as the polymerization degree increases *Ricardo-da-Silva et al. (1993).* As a consequence of browning, after clarification, the absorbance at 420 nm increases while decreases at 280 nm as confirmed by *Ricardo-da-Silva et al. (1993).*

Flavonoids reside mainly in grape skin, seeds, and stems. Their extraction increments especially after a prolonged contact of the juice with the solid parts. Extraction can also be enhanced through temperature or through the use of sulfur dioxide. Nevertheless, with a soft grape processing, the release of all phenolic compounds in the must, especially flavonoids, would be minimal and therefore the hyperoxygenation treatment would not have a significant effect on the sensory aspect. On the other side, non-flavonoids compounds are found not only in grape skins but also in the pulp cells vacuoles so they are the most present phenolics in white juices and wines where the contact with the skin and seeds is limited. However, those last compounds are not responsible for significant sensory aspects *(Ribéreau- Gayon et al., 2006; Schneider, 1998).*

Regarding the effect that exogenous oxygen supplies have on phenolic compounds in terms of color stability, few studies were taken into account. The purpouse was to evaluate if, as a general behavior, hyperoxygenation treatment could have a positive, negative or even no impact on the wines previously treated. An experiment done *by Cheynier et al. (1991),* consisted in supplying different amount of oxygen to musts coming from three different varieties, including Chardonnay. The average maximum oxygen consumption capacity was identified with 15 mg/L. The concentration of caftaric acid, coumaric acid, and GRP progressively decreased with the increasing amount of oxygen supplied. The wines under hyperoxygenation treatment were ranked at higher positions than the control wines concerning frankness and quality, with the highest scores for the wine treated with the maximum value of 15 mg/L of oxygen. The color, on the other hand, was better rated in the control wines for the higher intensity. No aroma or quality degradation was reported. Therefore, according to these results, both quality and aromatic

property were not significantly affected by the hyperoxygenation treatment. Conversely, wine quality improved, as confirmed in the previous study of *Cheynier et al. (1989)* which underlines how flavonoids are the main indicators of browning susceptibility, especially procyanidin B1. Another indicator suggested by the author is the glutathione - caftaric acid ratio, strictly dependant on the must extraction process and composition. In the experiment carried out by *Cheynier et al. (1989)* on Chardonnay musts, there were clear results of a decrease in all assayed phenolics concentration and browning potential in comparison to the control wines. The wines were browner after the pre-fermentative treatment and after fermentation, but they showed a general higher resistance to browning and a successive lighter color during the time spent in storage.

Table 2-1. Concentration of the main phenolic compounds subjected to oxidation in Chardonnay must of non-hyperoxygenated and gradually more hyperoxygenated musts (Cheynier et al., 1991).

Prefermentation treatment	Concentration of phenolic compounds (μM)		
	Caffeoyl tartaric acid	GRP[1])	p-Coumaroyl tartaric acid
Control	96.5	21.5	24.5
3.75 mg/l O_2	91.5	20.4	19.2
7.5 mg/l O_2	67.2	14.1	10.4
15 mg/l O_2	19.1	4.0	7.2

A different angle was given by the study of *Mayèn et al. (1996)*, focused on Sherry wine production with partial sludge removal instead of the complete elimination of the sediments after clarification. The experiment was carried out treating wines with or without SO_2 and/or O_2, in order to show the differences according to the use of one of the additions, both, or none. As shown in Table 2-2., the wines where only SO_2 was added, presented the lower value for polyphenolic fractions concentration. The untreated wines (without SO_2 or O_2), were the ones with the higher content of hydroxycinnamic acid fractions and catechin. Ultimately, the wines treated with oxygen (with or without SO_2 addition) presented the highest concentration of procyanidins, flavonols, hydroxycinnamic esters and hydroxybenzoic acids. The partial sludge removal caused a decrease in effectiveness with regards to the hyperoxygenation treatment compared to other studies where a complete removal after clarification was performed. It is not surprising data considering that most condensed polyphenols precipitate and are successively eliminate with the removal of the sludge *(Mayèn et al. 1996).*

Table 2-2. Changes in the percentage of the main polyphenol fractions before and after the treatments above mentioned. For each period and samples, the initial and final Student-t values and probability are shown *(Mayèn et al. (1996).*

Period	Treatments	Benzoics T(*P*)	Cinnamics T(*P*)	Esters T(*P*)	Catechins T(*P*)	Procyanidins T(*P*)	Flavonols T(*P*)
Initial—after O_2	with O_2–without SO_2	$+7.31^{***}$	$+6.66^{**}$	-8.57^{***}	-0.20	$+5.90^{**}$	$+1.33$
Initial—after SO_2	without O_2–with SO_2	$+5.74^{**}$	$+3.82^{**}$	$+11.4^{***}$	$+1.41$	-0.747	$+1.79$
	with O_2–with SO_2	$+4.24^{**}$	$+0.019$	$+2.80^{*}$	$+1.45$	-3.10^{*}	$+0.445$
	without O_2–without SO_2	$+5.08^{**}$	$+5.71^{**}$	$+21.1^{***}$	$+6.42^{**}$	$+22.1^{***}$	$+1.43$
Initial—end fermentation	without O_2–with SO_2	-1.27	$+2.24^{*}$	-3.67^{*}	$+3.70^{*}$	$+22.7^{***}$	$+3.27^{*}$
	with O_2–without SO_2	$+4.67^{**}$	$+7.61^{***}$	$+11.5^{***}$	$+10.2^{***}$	$+7.08^{**}$	-0.744
	with O_2–with SO_2	$+9.36^{***}$	$+6.16^{***}$	$+9.04^{***}$	$+12.8^{***}$	$+7.87^{***}$	$+0.940$
	without O_2–without SO_2	$+1.96$	$+2.05$	$+0.289$	-2.80^{*}	-2.48^{*}	-1.82
End fermentation—30 days	without O_2–with SO_2	-0.306	-3.01^{*}	$+2.19^{*}$	-6.25^{**}	-5.17^{**}	-6.63^{**}
	with O_2–without SO_2	$+0.258$	-2.46^{*}	$+0.328$	-6.27^{**}	-1.49	-1.45
	with O_2–with SO_2	-1.73	-1.16	$+0.010$	-3.14^{*}	$+0.278$	-1.34
	without O_2–without SO_2	$+6.10^{**}$	$+9.89^{***}$	$+10.9^{***}$	$+4.63^{**}$	$+95.8^{***}$	-1.41
Initial—30 days	without O_2–with SO_2	$+3.79^{**}$	$+8.65^{***}$	$+26.7^{***}$	-2.48^{*}	$+150^{***}$	-1.84
	with O_2–without SO_2	$+5.49^{**}$	$+3.99^{**}$	$+7.07^{**}$	$+2.95^{*}$	$+658^{***}$	-0.636
	with O_2–withSO_2	$+15.8^{***}$	$+9.17^{***}$	$+20.5^{***}$	$+2.80^{*}$	$+41.9^{**}$	$+1.43$

$^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

In disagreement with the previous results reported by *Mayèn et al. (1996), Ricardo-da-Silva et al. (1993)* found significant losses of GRP, cafaric acid, cis-coutaric and trans coutaric acids due to hyperoxygenation, on Grenache Blanc this time. As shown in Table 2-3., flavan-3-ols composition and concentration (dimeric and trimeric procyanidins, galloylated or not) presented some differences according to the pre-fermentative treatment. The total concentration was founded in higher amounts in those musts treated with pomace contact, whereas after hyperoxygenation, the loss of all procyanidins quantified was evident. Despite that, procyanidin B1 was the one present in highest concentation for all wines, with or without oxidation and/or pomace contact.

Table 2-3. Procyanidin composition in finished wines according to the pre-fermentative treatment performed (Ricardo-da-Silva et al., 1993).

Wines	Concentration of procyanidins (mg / L)[a]								
	B_1	B_2	B_3	B_4	C_1	T_2	$B_1$3-0-g	$B_2$3-0-g	$B_2$3'-0-g
Control (G)	1.22	0.13	0.02	0.01	0.22	0.19	0.02	0.04	traces
Hyperoxidation (GO)	traces	traces	traces	traces	traces	traces	traces	traces	traces
Maceration (GM)	3.00	0.32	0.15	0.24	0.27	0.51	0.05	0.10	0.02
Maceration+hyperoxidation (GMO)	0.07	traces	traces	traces	traces	traces	traces	traces	traces
Carbonic maceration (GCM)	11.92	1.21	1.23	0.20	0.50	2.49	0.18	0.08	0.06
Carbonic maceration + hyperoxidation (GCMO)	1.13	0.05	traces	traces	0.07	0.15	0.03	0.05	traces
Carbonic maceration press (GCMP)	24.10	1.46	2.05	0.44	1.39	6.31	0.28	0.17	0.10

[a] B_1 = procyanidin B_1; B_2 = procyanidin B_2; B_3 = procyanidin B_3; B_4 = procyanidin B_4; C_1 = procyanidin trimer C_1; T_2 = procyanidin trimer 2; B_1 3-0-g = procyanidin B_1 3-0-gallate; B_2 3-0-g = procyanidin B_2 3-0-gallate; B_2 3'-0-g = procyanidin B_2 3'-0-gallate.

Some authors like *Singleton et al. (1980)* believe that hyperoxygenation decreases excessively the aromatic and phenolic profile of the wine thus affecting its quality, whereas according to *Cheynier et al. (1989)* a moderate oxygenation can improve the organoleptic qualities, especially in relation to the quantitative and qualitative composition of the phenolic compounds founded in the grapes. The different opinions are not surprising and the motivations can be related to the variability of important factors. Factors such as the must composition and the amount of oxygen supplied, the eventuality or not of a previous skin contact treatment, or simply the batch, the clone or the state of the grapes at harvest.

An important point to take into account concerns the use of sulfur dioxide which reduce to a certain extent the effect of hyperoxygenation *(Mayèn et al., 1996).* It prevents o-quinones formations and their further polymerization and precipitation. Sulfur dioxide should not be avoided but the addition should be delayed until the clarification process is done, in order to avoid microbiological spoilage before and during fermentation.

2.3.2 Hyperoxygenation effects on volatile composition and sensory characteristics

The effect of oxygen on wine aromas mainly depends on the grape variety, the must composition and the quantity of oxygen supplied (*Zironi et al., 2010).* In musts, varietal aromas such as terpenes are mostly present in the glycosides form as aroma precursors. At that stage, it is harder for an oxidative reaction to happen because they are protected by the binding with the sugar (*Zironi et al., 2010).* At the wine stage instead, the glycosides are broken and the sensitivity to oxygen of the same molecule without the sugar increases, as a result of their conversion into the free form. Due to this reason, according to *Zironi et al. (2010),* a pre-fermentative hyperoxygenation would not significantly affect the expression of these aromas. In agreement to that, *Cheynier et al. (1991)* confirmed that the aroma profile can be preserved and the quality can even be improved.

Aromatic varieties such as Sauvignon Blanc are much more sensitive to oxygen. The sulfur-containing compounds, typical of Sauvignon Blanc, are in part bounded before alcoholic fermentation, thus being relatively protected (*Zironi et al., 2010).* Despite that, these sulfur compounds being susceptible to oxidation, they can be partially lost in the must as well, as suggested by the author. From this, the traditional way of working with these aromatic varieties under constant reductive conditions is more appropriate, in order to preserve the varietal aromas and the typicality of the wines.

Hyperoxydation was proven to cause some changes in the aroma composition of white wines by few authors whereas it didn't show significant differences in terms of aroma loss or quality by others.
Singleton et al. (1980) states that the oxygen addition provokes a lack of aroma intensity and a decrease of varietal aromas, leading to a loss of cultivar typicality. More recent studies from *Cejudo-Bastante et*

al. (2011) showed changes in aroma composition but they were not perceived as negative. The hyperoxygenated wines presented differences in comparison to the controls, with regards to the volatile composition, even after storage under both light and dark conditions. The concentration of isoamylic alcohols, acetaldehyde, β-damascenone, and flowery aroma decreased (Fig. 2-3.). On the other hand, isoamyl acetate concentration and consequently the banana aroma were evaluated as higher, together with the C6 alcohols content, giving an overall more intense freshness odor perception than the non treated wines. According *Cejudo-Bastante et al. (2011),* the oxygen favored the formation of high alcohols such as 2-phenylethanol and also C6 alcohols and aldehydes, such as 1-hexanol and 2-hexenal. The latter compounds, in fact, derives from their precursors linoleic and linolenic acids which synthesis is favored by oxygen. In disagreement to that, *Dubourdieu et al. (1990),* proved that the content of C6 alcohols decreased after hyperoxygenation in Semillion musts.
A decrease of the herbaceous aromas was noticed by *Cejudo-Bastante et al. (2011)* and the result is confirmed by *Nicolini (1992)* on Sauvignon Blanc wines. On the contrary, the concentration increased in a French white wine study from *Guedes de Pinho et al. (1994).*

Regarding the terpene concentration, *Dubourdieu et al. (1990)* reported no significant variation. On the other side, an increase of free terpenes was noticed in Penedès by *Artajona et al. (1990),* not only in Chardonnay wines but also in Muscat and Parellada. In the latter study it was also evident, for the hyperoxygenated wines, an increase of the fatty acids content and their esters and acetates of large chain alcohols. *Schneider (1994)* confirmed the same results in France and Germany. On the contrary, the paper of *Cejudo-Bastante et al. (2011)* asserts that floral aroma decreased in treated wines, probably in relation to the decrease of the β-damascenone concentration. Fruitiness improved thanks to the oxygen addition and the given explanation was the direction of the yeast metabolism during fermentation towards the production of short- and medium-chainn fatty acid esters over higher alcohols. A previous study from *Schneider (1996)* on Riesling wines instead, reported a decrease of the peach aroma and an increase of the lemon aroma after hyperoxygenation.

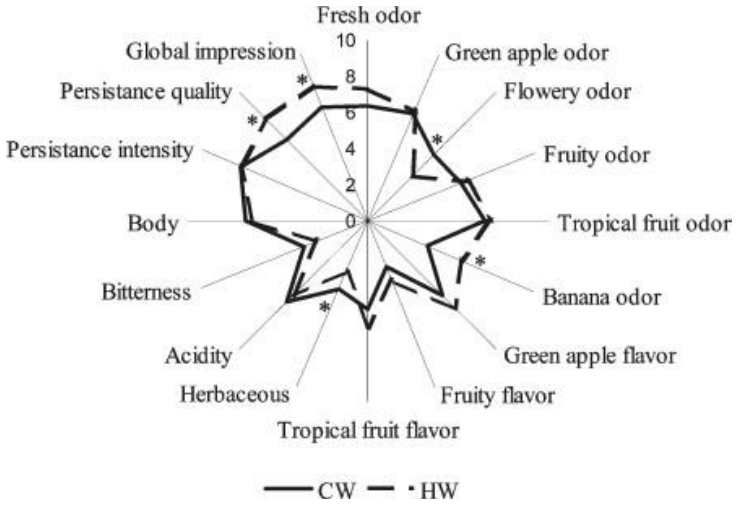


Figure 2-3. Differences in olfactive and gustative attributes for non treated Chardonnay wines (CW) and Hyperoxygenated Chardonnay (HW). The scores here represented indicates the mean values presenting significant differences according to the Student's t-test ($p<0,05$) (Cejudo-Bastante et al., 2011).

To summarize, according to most of the studies, the wines under hyperoxygenation treatment did not show a significant loss in quality and aroma; on the contrary, they were better rated on most cases which is an interesting background for further studies on the topic. Considering the information and the differences within the results found in the literature, specifics on the hyperoxygenation conditions should be determined according to each must in particular. It can be helpful after an accidental pomace contact occurs, to decrease or prevent the browning effect or to smooth the effects of an excessive extraction. According to the winemaking style and the variety and maturity of the grapes, it might be a useful pre-fermentative treatment for the future quality of the wine, without affecting significantly the organoleptic profiles but reestablishing the balance between oxygen and flavonoids, especially when the content of the latter ones is too high to be eliminated by the oxygen naturally available. However, it should be avoided in some other situations where the initial phenolic content is already low.

2.4 How hyperoxygenation can be performed

There are few different ways to perform hyperoxygenation on musts, depending on the available equipment. The aim is to reach oxygen saturation after the pressing and before clarification and fermentation. According to *Schneider (1998),* saturation is considered to be reached at 9 mg/L of oxygen under the temperature of 15°C degrees. The author showed in his work some of the possible means to perform this technique:

i. Oxygen can be provided through diffusers, while the must is transferred from tank to tank, from the press into a tank or directly in a tank through the gas hose while stirring the juice. In the first case, the gas flow rate must be adjusted with the must flow rate to achieve for example a value of 10 mg/L of dissolved oxygen in the must. If further saturation is needed it would be necessary to wait until part of the dissolved gas would have been used.

ii. In case the must is clarified by flotation, the two operations can run at the same time. Nitrogen would be replaced by oxygen and finally, all the phenolic precipitates will be removed. In this case, the gas uptake would be over the saturation concentration level, thus causing maximum flavonoid precipitation.

iii. In a small production scale, the objective can be reached simply aerating the must from bucket to bucket until obtaining a dark brown color.

The bubble's size is directly responsible for the gas dissolution time. The bigger the size, the higher the diffusion rate. The porosity of the diffusers determines that size, which should be equal or inferior to 0,003 mm and would automatically increase when organic deposits are found on the device's surface. Using hermetically closed experimental devices allows better monitoring of the oxygen needed and consumed (*Schneider, 1998).* In all the other cases is much harder to establish the accurate value, especially on an industrial scale.

In the work of *Cheynier et al. (1991)* an experimental device was used to hyperoxygenate the must under constant must and gas flow rate, adding the desired amounts of oxygen until completion. The gas was diffused while the must was circulating through the tank and pipes. The control on the experiment duration allowed reaching a different amount of oxygen supplies, which consumption was controlled by of a WTW Ox 91 oximeter equipped with a Clark electrode. During a previous experiment instead, the oxygen was added through a diffuser at the beginning of a transfer pipe while the must be pumped from one tank to another. Both flow rates of must and oxygen were calculated with the aim of supply 16 mg/L of oxygen *(Cheynier et al., 1989).* Contrarily, values of 20-30 mg/L of oxygen were reported by *Schneider (1998)* and considered to be more than sufficient to cause efficient precipitation of flavonoids.

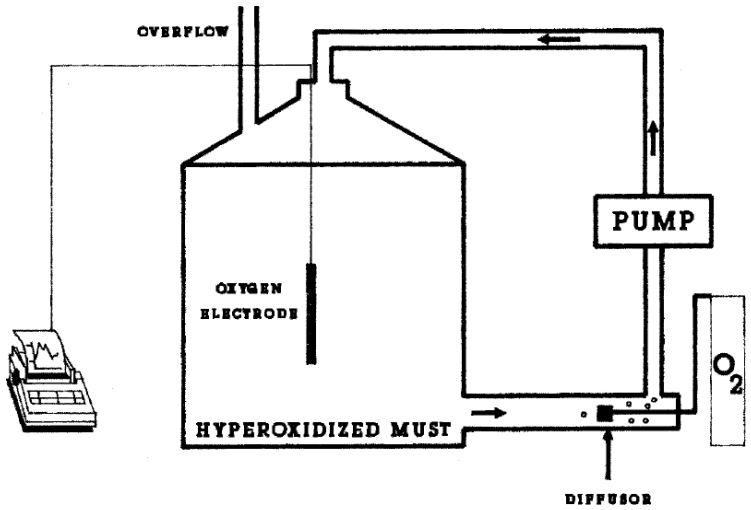


Figure 2-4. Hyperoxigenation experimental device used by *Cheynier et al. (1991).*

In the experiment described by *Ricardo-da-Silva et al. (1993*), the oxygen was added through a diffuser into the must, pumped from a tank to another after pressing. In order to achieve a better oxygen homogenization, the addition was made at the beginning of the transfer pipe and the objective was to reach the total amount of 50 mg/L of oxygen supply per must. This amount was evaluated to be consumed within the time of six hours and the monitorization of the concentration was performed using a WTW Ox 91 oximeter equipped with a Clark electrode. Sulfur dioxide was successively added in the concentration of 30 mg/L in order to better stabilize the must. On the other side, as reported by *Cejudo-Bastante et al. (2011),* the same amount of 50 mg/L was supplied to the must but in 4.5 hours. It was used a silicon diffuser connected to a cylinder with the objective of pumping the oxygen from the bottom to the top of the tank, using a Laffort oximeter for flow control.

Different amount of oxygen supply was reported in the literature but, as previously mentioned, the content of oxygen required depends on the must composition, especially with regards to the initial hydroxycinnamic acids content and the hydroxycinnamic acids glutathione molar ratio which defines the oxygen consumption capacity *(Cheynier et al., 1990).* Eventual skin contact or drastic pressingalso has a big influence on the consumption capacity *(Moutounet et al., 1990).*

2.5 Chardonnay typicality

2.5.1 The concept of cultivar typicality

"A wine is typical if some of its own characteristics can be identified and make it recognizable as belonging to a type and distinctive from others*" (Maitre et al., 2010).* "The typicality concept is

supported by the existence of a common memorized prototype which represents the image of all the previous experiences of wines from the type*" (Casabianca et al., 2005).* These two defnitions give a good idea of what typicality is. The tool used to assess a wine typicality, defined as perceived representativeness by *Cadot et al. (2010),* is sensory analysis. The memory of a common prototype, in this case, the wine's cultivar, is what defines the wine typicality and supports the representation of all the previous experiences of that wine's cultivar by the tasters.

Wines can be defined by winemaking techniques, type of soil, vintage, but especially by the specific cultivar is made out from. Varietal aromas make the identification much easier, they allow the tasters to build a mental prototype correlated with the specific cultivar, for instance associating methoxypyrazines with Cabernet Sauvignon. Onthe other hand, more neutral varieties give a harder time to be recognized if the tasters are not well trained or experienced. It is definitely not easy to assess typicality or to define the proper way to do it. The judgment strictly depends on the experience, sometimes it is influenced by a hedonic point of view *(Ballester, 2008*) and it is generally not objectively measurable by specific dimensions *(Ballester, 2004).*

According to *Maitre et al. (2010),* there are three types of tasters who should define typicality: technicians, users, and keepers of memory such as retired people from the sector. The categorization of the product can be done through two approaches: knowledge and similarity *(Lelièvre, 2010).* The first is adopted by professionals and experts and it is sometimes linked to a geographical aspect, whereas the second is adopted by the general consumer who simply recognize the product they are tasting. Remembering and associating specific characteristics lead to the possible identification of the product, in this case, the wine.

2.5.2 Scientifical methodologies for typicality evaluation

Different scientific approaches have been used to evaluate typicality or simply the degree of representativeness of a type of wine or a general product. Some of the most used sensory methods found in the literature and presented by *Cadot et al., (2010)* are:

i. Quantitative Descriptive Analysis (QDA): generally conducted by an expert panel, it provides a complete word description for all the sensory properties of the product.

ii. Just About Right analysis (JAR) by wine experts, it is based on the deviation from the ideal levels of the intensity measured per attribute. It allows also an overall liking evaluation.

iii. Assessment of the typicality, evaluated mostly by wine experts. Panelists have to rate the sample's belonging degree to a certain category, using a scale going from "bad example" to "good example".

A typicality assessment could be approached in two different ways: by categorization or rating. Categorization based on similarity is generally used for consumers because it doesn't require a high level of expertise and it's well related to everyday life. The tasters are asked to make a comparison between the given sample and the category, being influenced by their personal experience. The issue is that it requires a very good representative sample to be used as a reference, as stated by *Ballester et al. (2005)* in their work on the "Chardonnay wine concept". The panel can be asked if the sample matched their concept or not and how sure they are of their answers.

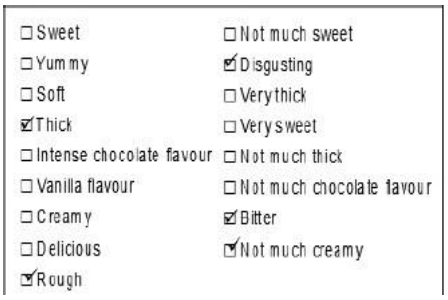
A representativeness rating, on the other hand, can be done asking the panel to rate on a given scale the intensity related to a specific attribute. In this case, experts are the most qualified for this task because it requires at least some training *(Ballester et al., 2005).*

"Scaling" is an immediate and useful way to get intensity or liking information. It is used to describe a judgment or, in this case, the typicality of a cultivar which will be translated later on into a numerical value, converting the responses in regard of intensity/strength into quantitative data. Within the Descriptive analysis methods (DA) scaling is considered to be part of the basis. Line scaling for intensity is a widely used technique consisting is making a mark on a draw line in order to evaluate the intensity of a specific attribute. The distance from one end of the scale to the mark is the response in the form of a number. Generally, the endpoints of the line are labeled and short line segments are positioned close to both extremities indicating the lowest perception of that attribute and the highest, even if further intermediate points can be added. This is generally done to reduce the reluctance effect that some people have to use the ends of the scale in the eventuality that a more or less intensity of that attribute will appear later on with another product. Vague terms to describe the attribute should generally be avoided, taking into consideration the training level of the panelists as well. The degree of variability in the scale among judges it depends on a common or not common frame of references the tasters share about that attribute *(Lawless, 2010).*

Martin et al. (1992) used a five-point scale to evaluate the typicality of flor Scherry wines, while *Moio et al. (1993)* used a three-point scale to assess wine typicality for Burgundy Chardonnay wines. Another way for this type of evaluation is rating the samples on a scale ranging from very typical to a non-typical, instead of using points. The results will provide a gradient of a good and bad example of Chardonnay typicality, exactly as it was done by *Jaffrè et al. (2011).*

Once the wine's typicality degree has been evaluated through rating, the focus should be pointed on describing the sensory profile of either that specific cultivar or that type of wine. Recently, more sensory methodologies have been used and they are known as rapid methods. They are considered to be easier to perform on a practical point of view for both the experimenter and the tasters, being less time consuming and less tiring for the assessors. An appropriate method to generally describe a product is the CATA analysis ("check-all-that-apply") in order to define what could be the most frequent and suitable profile of the wines rated as the most typical ones.

CATA is a verbal-based rapid method used to describe a product, allowing the tasters to choose the attributes they find more appropriate within a provided list. Following a balanced or randomized design, the products are evaluated one by one by the members of the panel. A key point is the selection and the number of the attributes chosen for the list. Generally, a short one gives better results in terms of description efficiency by the panelists. There is no limit on the attributes that can be checked and each attribute will be counted in order to establish the frequency of its use for the wine description. Once the frequency matrix is compiled, further analysis can be performed, such as Correspondence Analysis (CA) in order to build sensory maps *(Valentin et al., 2012).*



Product	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17
1	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1
2	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	1	0
4	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0
5	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1
6	0	0	0	0	1	0	1	0	1	1	0	1	0	0	0	1	0
7	0	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	1	1	0	0	1	0	0	0	1	1	0	0

Figure 2-5. Example of a CATA list of attributes and the data coding where each checked attribute (from A1 to A17) is indicated with the value of one. All the remain attributes are instead identified with the value of zero (Valentin et al., 2012).

CATA is an efficient method to discriminate samples and its simplicity make it very useful on a practical point of view, compared to Descriptive Analysis, the more conventional sensory method *(Valentin et al., 2012)*. CATA can be performed with or without trained assessors while DA requires the training of the panelists. This is one of the reasons why the last one is considered to be more accurate. CATA doesn't take into account intensity or ranking but only the frequencies of how often each attribute was chosen. Furthermore, it requires a larger number of tasters *(Valentin et al., 2012)* while DA generally needs between 8-12 panelists previously trained who will use a quantitative scale for intensity, in order

to proceed with the statistical analysis of the data. The aim, in this case, is to obtain precise indications of how a product differs from another one in the sensory dimension *(Lawless, 2010).*

DA would be a better option to highlight small differences regarding, for instance, the intensity of specific attributes on a sensory point of view. However, to assests a wine typicality and to obtain a sensory profile of the wines, the Scaling method combined with the CATA evaluation can well adapt to the objective.

2.5.3 Chardonnay aroma profile in correlation with typicality

Contrarily to aromatic varieties such as the Muscat family, Chardonnay's aroma profile is not as immediate to determine, mainly because it is not defined by varietal volatiles as easy to recognize as in case of aromatic cultivars. Despite that, there are still many compounds able to describe and identify this cultivar in order to assess wine typicality, according to their presence and concentration.

Different chemical families contribute to the Chardonnay aroma profile:

i. C13-norisoprenoids: mainly β-damascenone (described as “lemon balm”, “apple”, “rose”, “honey”, according to the concentrations) which has a very low threshold (50 ng/L) making it a powerful odorant *(Gambetta et al., 2014).* Other possible descriptors for this molecule are: “baked apple”, “canned peach” and “dry plum” *(Li et al., 2008).* Vitispirane and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) (described as “kerosene”, generally typical of aged Riesling) are also good contributors to the varietal aroma *(Gambetta et al., 2014).* Finally, 3-oxo-a-ionol, at the concentrations found in Chardonnay wines, was described as “spicy” by *Lee et al. (2003).*

ii. Thiols: especially benzenemethanethiol (BM) (threshold: 0.3 ng/L) which contributes to the mineral character with its "flinty" notes. It was identified as the compound having its highest concentration in Chardonnay wines, between many other varieties analyzed *(Gambetta et al., 2014).*

iii. Esters: one of the most significant categories for the aroma profile of this cultivar, giving the characteristic floral and fruity notes *(Gambetta et al., 2014).* The most powerful odorant esters found in Chardonnay wines by *Li et al. (2007)* are: ethyl octanoate (described as “pineapple”, “floral”, “pear”), ethyl butyrate (described as “sour fruit”, “fruity strawberry”), ethyl decanoate (described as “fruity”, “pleasant”, “fatty”), ethyl lactate (described as “lactic”) and ethyl hexanoate (described as “fruity”, “green apple”, “strawberry”).

iv. Higher alcohols: together with the esters, these are compounds produced during alcoholic fermentation thought the yeast metabolism. The most significant ones for this cultivar are 2-phenyl-ethanol ("flowery aroma") and iso-pentyl alcohol ("bitter", "alcohol", "harsh", not positive overall). Iso-butanol and 1-propanol can also contribute but on a lower extent *(Li et al.,2008).*

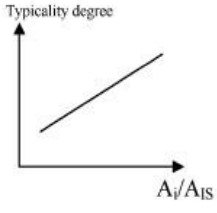
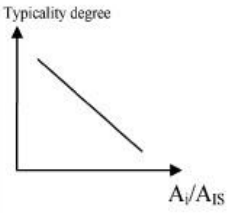
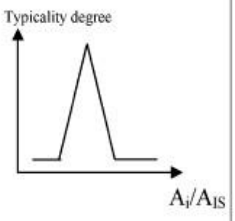
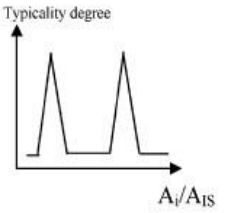
v. Fatty acids: mainly octanoic and decanoic acids which do not have a positive effect on the general aroma. Hexanoic acid, in addition, is characterized by "cheese" notes *(Li et al., 2008).*

Not all volatile compounds can be perceived and will contribute to the final aroma. Their concentration, together with their threshold level, defines the power of their impact and therefore their perception, making that compount more or less characteristic for the wine. *(Gambetta et al., 2014).* The main odorants contributing to a typical Chardonnay wine aroma profile according to the same author are listed in Tab. 2-5.

Assuming that typicality derives from an association of specific proportions of specific volatiles, an experiment was carried out by *Lorrain et al. (2006)* in order to confirm the contribution of certain target compounds on Chardonnay typicality. A sensory evaluation by orthonasal perception was performed after target compounds were identified in 20 wines. The methodology used is the one previously developed by *Ballester et al. (2005)* and *Candelon et al. (2004).*

Some of the wines with an intermediate typicality score were supplemented with 6 or 10 compounds combinations. Finally, their typicality degree was assessed. The results showed how the wines supplemented with 10 compounds reached higher scores of typicality degree, thus becoming more representative of the cultivar. Whereas the ones supplemented by 6 compounds only, remained into the intermediate range.

Table 2-4. Correlation of 23 different compounds with wine typicality, showing four patterns of relationship between the typicality degree of the wine and the relative compounds' concentrations (A/A_{IS}) *(Lorrain at al., 2006).*

Categories	Positive	Negative	Single optimum	Double optimum
Four patterns of relationship between typicality degree and A_i/A_{IS}				
Compounds	ethyl butanoate ethyl hexanoate δ-decalactone 2-hydroxy-4-pyranone 4-vinylphenol 2-methyltetrahydrothiophen-3-one 4-vinyl-2-methoxy-phenol linalool	vanillin α-terpineol ethyl furoate ethyl 2-methyl butanoate diethyl pentanedioate ethyl 3-methyl butanoate 1,1-diethoxy ethane	benzyl alcohol diethyl butanedioate phenylacetic acid ethyl hydrogen succinate	3-methylbutyl acetate phenylacetaldehyde octanoic acid decanoic acid

According to the study of *Lorrain et al. (2006)*, 23 compounds were found to be connected with the typicality degree, in relation not only to their presence but especially to the specific concentrations. The major descriptors for the positive correlations were: fruity, floral, microbiological, spicy, chemical and nutty. Except for the negative correlation, all compounds belonging to the other three cases were defined as the major descriptors for Chardonnay wine typicality in this experiment. Clearly, a more complex model could enhance the typicality degree of the wines. An oversimplified one was not able to fully express the main characteristics of the variety. The complexity of the model and the choice of the panelist together with their level of training also have a big influence on the results.

Ballester et al. (2005) worked on demonstrating a shared Chardonnay wine sensory concept, consensual among experts and referred to Chardonnay wines coming from different French wine regions. The French experts agreed in assessing the representativeness of the wines during a tasting including different cultivars as well, in order to demonstrate the efficiency of the rating approach and the variety typicality.

To support the idea of chemical odorants responsible for the tipicality of the wine coming from a specific cultivar and therefore not affected by the geographical location, soil and climate, it is necessary to point out that the grapes used for the experiment by *Lorrain at al. (2006)* came from France while the data on which the experiment of *Gambetta et al. (2014)* was based are related to a previous study in Spain where the grapes were harvested. Some of the compounds defined as typical are common in both studies: the esters ethyl butyrate and ethyl hexanoate and the fatty acids octanoic acid and n-decanoic acid. The same compounds were analyzed and defined to be the most powerful impact odorants in Chardonnay wines

in North China as well, by *Li et al. (2007).* In addition to that, β-damascenone, phenethyl acetate, ethyl octanoate, ethyl lactate, ethyl decanoate, 2-phenyl-ethanol, and hexanoic acid were found to be other major representative compounds in both the Chinese and the Spanish studies. Furthermore, from the experiment of *Louw et al. (2010)*. The main volatile compounds responsible for distinguishing Chardonnay wines were considered to be: ethyl octanoate, hexyl acetate, 2-phenylethanol, diethyl succinate, decanoic acid, ethyl decanoate, 1-propanol, and ethyl hexanoate.

The origin of the grapes and both viticolture and enological practices, seems to not cause an over-writing of the cultivar typicality. The grapes used for the experiments above mentioned, in fact, came from different regions and countries but reported a similar outcome with regards of the aroma profile for Chardonnay wines. Therefore, it is possible to suggest that the above-listed compounds are good representatives for the Chardonnay aroma profile derived from the cultivar itself. The same statement, based on the studies of *Lorrain et al. (2006)* and *Ballester et al. (2005),* was done by *Jaffrè et al. (2011),* confirming the existence of an olfactory space for young unoaked Chardonnay wines, not dependant on vintage or geographical origin. In this last case 35 volatile compounds out of the 62 analyzed, were found to play a role in the typicality evaluation but only 18 of them were considered to be the real responsible for it. The same 18 compounds were found by *Ballester (2004)* to have the same predominant role in determining Chandonnay typicality, as shown in the table below, as a resume of all the experiments mentioned in this section.

Table 2-5. Summarized list of the main odorants responsible for Chardonnay typicality according to the following studies, already mentioned in this paragraph. The compounds are listed in order of frequency of mention by the authors. The descriptors noted as "1" belong to the experiment of *Lorrain et al. (2006)*, the number "2" is related to the study of *Li et al. (2008)* while the number "3" to the one by *Gambetta et al. (2014).*

Impact odorants for Chardonnay typicality	**Descriptors**	**Ballester (2004)**	**Lorrain et al. (2006)**	**Li et al. (2008)**	**Louw et al. (2010)**	**Jaffrè et al. (2011)**	**Gambetta et al. (2014)**
ethyl hexanoate	fruity, pineapple, over-ripe fruit, anise [1], green apple, strawberry [3]	x	x	x	x	x	x
decanoic acid	dusty, fatty, waxy, synthetic [1], vinegar, animal [3]	x	x	x	x	x	x
diethyl succinate	wine, herbaceous, floral, fabric [1], caramel [1-3]	x	x	x	x	x	x
ethyl butanoate	fruity, strawberry [1-3], banana, pineapple, sweet, strawberry candy [1]	x	x	x		x	x
octanoic acid	cheese, fatty, acid, sweet, goat, rancid, unpleasant [1], animal, spicy, cheese [3]	x	x	x		x	x
benzyl alcohol	sweet, fruity, boiled cherry, roasted, moldy, herbaceous [1], floral [3]	x	x			x	x
Linalool	fruity, citrus [1-3], floral, sweet, lemon, herbaceous [1]	x	x			x	x
delta-decalactone	nutty, peach [1], coconut, floral [1-3]	x	x			x	x
ethyl decanoate	fruity, pleasant, fatty [2], oily, floral [3]			x	x		x
2-phenyl-ethanol	flowery, perfume, pollen [2], rose [3]			x	x		x
β-damascenone	stewed fruit, apple, peach [3], backed apple, canned peach, dry plum, bark [2]			x		x	x
ethyl octanoate	pineapple, pear, floral [2], fruity, sweet [3]			x	x		x
hexyl acetate	fruity, pear, pleasant [2], apple [3]			x	x		x
Phenylacetaldehyde	green, honey, floral, spicy [3]	x	x			x	
4-vinylphenol	spicy, pharmaceutical, phenolic, vanilla[1]	x	x			x	
4-vinyl guaiacol	smoke, phenolic [3]	x				x	x
alfa-terpineol	floral, musty, orange [3]	x		x		x	
hexanoic acid	rancid [2-3], cheese [2], pungent, green [3]			x			x
phenethyl acetate	floral, pleasant [2]			x			x
ethyl lactate	raspberry, lactic [2]			x			x
1-propanol	alcohol, fresh [2], ripe fruit [3]				x		x
2-methyltetrahydrothiophen-3-one	chemical, gas, diesel oil [1], metallic, natural gas [3]		x			x	
2-phenylethyl acetate	floral, rose [3]					x	x
1-butanol	medicinal, alcohol [2]			x			x
1-hexanol	grass, green [2]			x			x
TDN	kerosene, petrol [3]					x	x
Vanillin	vanilla [3]	x				x	
dodecanoic acid	dry, metallic, laurel oil [2]			x			x
3-oxo-a-ionol	spicy [3]						x

2.5.4 Hyperoxygenation influence on typicality

In the literature, there is a lack of information regarding a correlation between hyperoxygenation and typicality. From here the intention of investigating on the topic, performing the experiment described in this thesis project with the main focus on detecting the possible effects that hyperoxygenation might have on the typical aroma profile of Chardonnay wines. According to the results, an idea should be given concerning the possibility of using this treatment as a potential and worthy alternative to standard vinifications in case no significant changes will be detected. This will provide a similar product with a lower content of sulfur dioxide used during the vinification process and especially a reduced risk of browning during storage. It needs to be taken into account though, that hyperoxygenation could also excessively modify the wine, thus changing the sensory profile and creating a different wine style, which could be seen either as a positive outcome but also as a negative one, by some producers and especially consumers who want to recognize the product they are tasting.

3 MATERIALS AND METHODS

3.1 Small scale vinification

Grapes from *Vitis Vinifera* var. Chardonnay were harvested in a good sanitary condition and manually picked in two separate vineyards in Paarl (South Africa). The first batch (C1) was harvested on the 12th of February 2019 while the second one (C2) on the 20th February 2019. Each batch consisted of about 15 crates of 20 kg maximum capacity. A total of 300 kg per batch were processed in the experimental wine cellar at Stellenbosch University, after being left overnight at 4°C. Out of the 300 kg, 150 kg were processed under the standard condition and avoiding the contact with oxygen (control, C) while the other half was treated with a pre-fermentative hyperoxygenation (O) (Figure 3-2.).

In both cases, the grapes were destemmed and crushed using a CDS Vintec crusher-destemmer with the capacity of 0,5 ton-/h. The mash was separated into two groups for the different vinification process (C for the control and O for the Hyperoxygenated). Sulfur dioxide (30 ppm) was added after crushing only to the control juice. No addition was made for the juices destined to hyperoxygenation to avoid SO_2 interference with phenolic oxidation and precipitation.

Pressing was performed through a manual basket press with a capacity of about 150 kg. Carbon dioxide was used as an inert gas to avoid the contact with oxygen for the control musts, while for the treated ones no gas was utilized. The must was successively homogenized and divided to obtain three replicates per treatment (A, B, C). Six buckets per batch were filled, with a capacity of 20 L each.

Due to logistical constraints, especially oxygen dosing tools, the hyperoxygenation was performed through simple manual aeration of the must. The juice was poured from bucket to bucket until the color reached an intense brown tone. Afterwards, it was left in contact with the air for about 1 h.

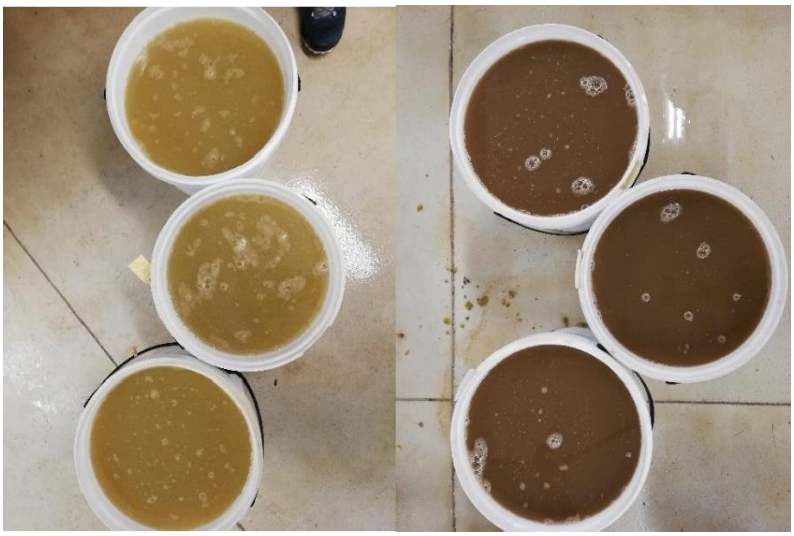


Figure 3-1. Pictures taken during the hyperoxygenation treatment. On le left the control must and on the right the hyperoxygenated must.

For better juice clarification, 1,5 g/HL of pectolytic enzyme LAFAZYM EXTRACT from Laffort was added to each one of the 20 L buckets, following the recommended dose of 0,5-2 g/HL.

The must was left overnight at 4°C and racked the next day to remove sediments and especially the oxidized brown phenolics. Each bucket was racked into a canister of 25 L capacity, to perform alcoholic fermentation separately. At this point, 30 ppm of SO_2 was added to the hyperoxygenated juices, to prevent microbiological spoilage.

Juices were inoculated with 20 g/HL of ANCHOR VIN13 (*Saccharomyces Cerevisiae*) and left at 15° for the alcoholic fermentation (AF). Fermentation was monitored following the decrease in weight of each canister to avoid any oxygen contact with the must from this stage on.

Once AF was completed (resudual sugar <2 g/L), a dose of 25 g/HL of a 5% bentonite suspension was added to clarify the wine. The wines were left at minus 4°C for cold stabilization for 3 weeks. Afterwards, SO_2 content was measured and adjusted to reach 30 ppm free SO_2 for each wine. After 24 h, the wine was racked under inert condition using CO_2. The wine was bottled right after using nitrogen to avoid oxidation. No filtration was performed on the wines. All bottles were stored at 15°C for about 2 months before the sensory analysis.

Samples were taken during vinification, on the finished wines and just before performing the sensory evaluation. All parameters analyzed for each stage are reported in the experimental layout in Fig. 3-2.

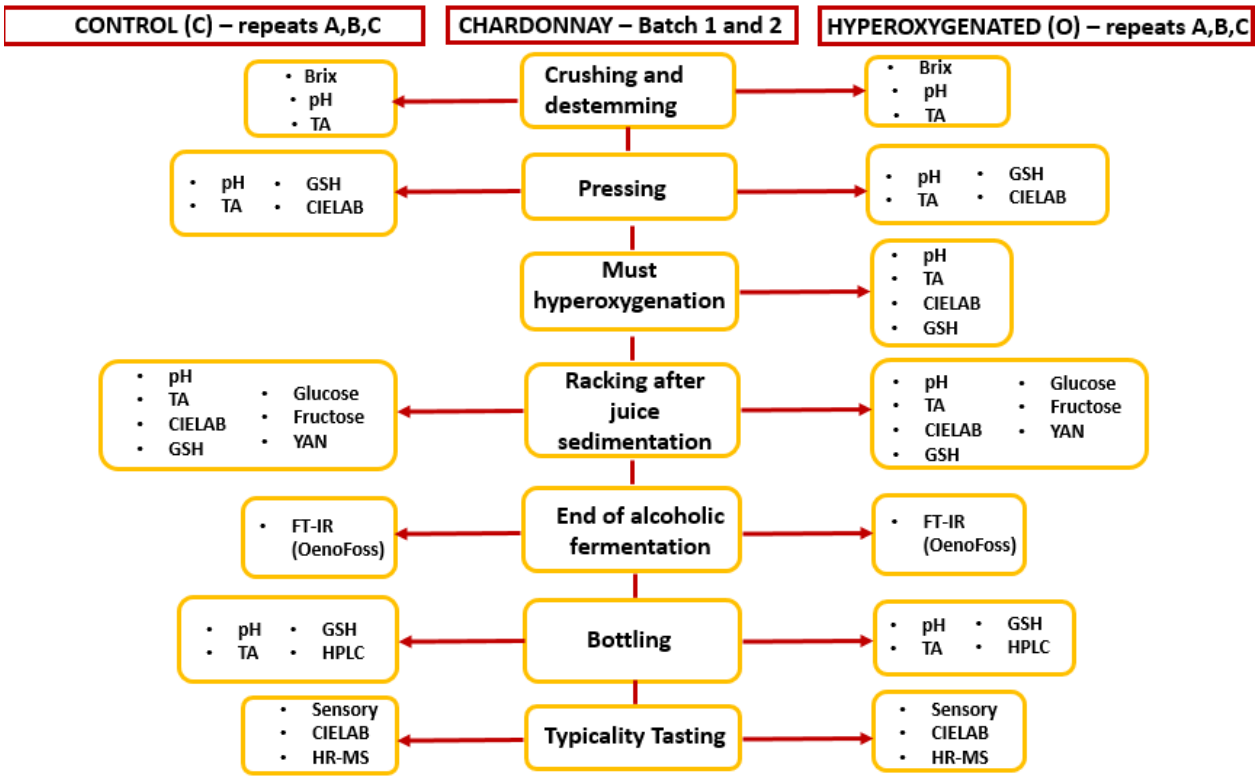


Figure 3-2. Experimental layout representing a summary of all samples taken and analysis performed during the winemaking process and the day of the sensory evaluation.

3.2 Chemical analysis

3.2.1 Standard enological parameters and sampling

The initial sugar content of the grapes was evaluated with the use of a hydrometer, measuring the relative density of the must after crushing the grapes. The Brix scale was used.

After must settling and racking, juice samples were sent to VinLAB, an independent laboratory in Stellenbosch, South Africa (ISO17025 and BBBEE accredited). The analysis was performed for glucose and fructose content (g/L) and the measurement of yeast assimilable nitrogen (YAN), for both free amino nitrogen (FAN) and ammonium (NH_4^+).

Fermentation was monitored measuring the weight of each canister daily. Once the weight remained stable for more than two days, a Fourier transform mid-infrared spectroscopy (FT-MIR) (WineScan FT 120, Foss Analytical, Denmark) was used to analyze the residual sugar content and confirm the end of the alcoholic fermentation. The wine was considered to be dry at less than 2 g/L of total sugars.

The pH, titrable acidity (TA) and free sulfur dioxide (FSO_2) of juices and wine samples were measured with a potentiometric titrator, type 702 SM Titrino (Metrohm Ltd., Switzerland).

At bottling, a high performance liquid chromatographic method (HPLC) was used to profile ethanol (g/L and % v/v), glycerol (g/L), major sugars (g/L) (sucrose, glucose and fructose) and organic acids (g/L) (tartaric, malic, citric, succinic, lactic, and acetic acid). The instrumental method was based on an Agilent product guide (5990-8801EN) with some modifications. The column was a Hi-Plex H (300 x 7.7 mm) ion-exchange with 10 mM sulphuric acid as mobile phase. A flow rate of 0.6 mL/min and a column temperature of 45°C were set. Both standards and samples were prepared following the method of *Eyéghé-Bickong et al.* (2012). The analysis was performed in duplicate for each standard, to have a better calibration, while only one injection was performed for each sample. An aliquot of 800 µL of deionized water was added to a 200 µL of wine sample. To reach a final volume of 2 mL and obtain a 10-time dilution, 1 mL of IS stock solution (4 g/L Ribitol and Adipic acid) was added. After vortexing and centrifuging the samples, HPLC vials were filled with 1 mL sample for the analysis.

3.2.2 Glutathione analysis

Glutathione (GSH) concentration, in both its oxidized and reduced forms, was analyzed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis, according to the method published by *Kritzinger et al*. (2013). The analysis on musts and wines was done on a Waters Acquity UPLC fitted to a Waters Xevo triple-quadrupole mass detection in positive mode (Milford, MA, USA). Each sample and calibration standard was diluted five times with a dilution solvent of HPLC graded Milli-Q-Water with 1000 mg/L SO2 and 500 mg/L ascorbic acid. The aliquot of 200 µL of homogenized juice or wine sample (or calibration standard) was then added to the solution. The diluted sample was centrifuged at 10000 rpm for five minutes (Centrifuge 5415 D, Eppendorf, Hamburg, Germany). Finally, 900 µL were transferred into a marked HPLC vial and capped.

3.2.3 Colorimetric measurements

The color was evaluated with a Thermo Scientific Multiskan GO 1510-02586 microplate spectrophotometer. Measurements were performed in triplicate following the protocol by OIV (2006) with some modifications. Due to some limits of the machine, a reduced spectrum was used. Instead of measuring the absorbance from 380 nm to 780 nm, it was measured only between 380 nm and 600 nm. The parameter determined were the following: total phenolics (A280), hydroxycinnamic acids (A320), flavanoids (A360) and yellow pigment, indicator of browning (A420).

Chromatic characteristics were calculated according to the method of OIV (2006) from the Commission Internationale de l'Eclairage (CIELab), optimized for white wines. The colorimetric coordinates derived from the calculation are clarity (L*), green/red color component (a*), blue/yellow color component (b*), chroma (C*) and tone (H*).

3.3 Sensory evaluation

The present work was subjected to ethical clearance from Research Ethics Committee: Humanities, protocol number: VIT-2019-10195.
Before undergoing sensory analysis, the wines were stored at 15°C for two months. The evaluation was performed at the Sensory Laboratory of the Department of Viticulture and Oenology, Stellenbosch University on the 6th of June 2019.

Instructions were given to twenty expert judges and the evaluation was completed in one session. One single flight of 12 samples was presented with both Chardonnay batches. Each batch included 3 repeats for the control wines and 3 repeats for the hyperoxygenated wines. All samples were presented in a randomized order. Each sample was coded and identified with unique three-digit numbers generated by Compusense® five computer software (Release 5.6), used for the experimental design.

Figure 3-3. The picture was taken before the sensory analysis. Twelve samples of Chardonnay wines were tasted: two batches, two treatments and three repeats per treatment.

The typicality of the wines was rated using an unstructured scale, as described by *Lawless (2010).* Judges were asked to evaluate how much the wines were representative for the Chardonnay cultivar. Shortline segments were positioned close to both extremities of the scale, indicating the lowest and the highest perception of typicality. The segments were marked as “bad example” on the left and “good example” on the right.

Additionally, the aroma profile of the wines was described through a rapid method, CATA ("check-all-that-apply") according to the protocol described by *Valentin et al., (2012).* For the odor description, a pre-determined list of attributes was given to the judges. The panel was asked to select as many attributes they find to be appropriate to characterize the aroma of each sample. For the taste description, the intensity for sweetness, sourness, body, astringency, bitterness, and length was assessed. Five possibilities were given to the judges ranging from “absent” to “very high” regarding the intensity perception for each of the 6 attributes. An example of both tasting sheets used for the sensory analysis is reported in the Appendix.

For the aroma profile, a contingency table with the wines in the rows and the aroma descriptors in the columns was compiled for the CATA data. In case the frequency of citation for a certain descriptor was counted less than three times between all judges, the descriptor was combined with another one of the same family that shared a similar meaning. Throughout the study, the rules for this combination were kept constant. Citation of each descriptor was finally summed up and the results indicate the frequency of use of that specific attribute by the panel.

3.4 Statistical Analysis

For statistical data analysis of the typicality rating, Microsoft Word with XLSTAT (Version 18.06, Addinsoft) was used. Differences on typicality between the control and the hyperoxygenated wines were tested for significance by applying the analysis of variance (one-way ANOVA). The p-value threshold was established at 0,05 to determine the statistical significance. To check the validity of the results, a normality test was run first, followed by a Levene’s test for the equality of variances. A non-parametric test (Kruskal Wallis) was then used, the population being not normally distributed.

Correspondence Analysis (CA) was performed on sensory data from the CATA evaluation using the frequency of citation. CA is a multivariate method to investigate the association between variables on a symmetric and correspondence point of view *(Jaeger et al., 2015; Vidal et al., 2015*). Furthermore, to find out similarities and differences between the wine samples and the treatments/batches, a multi-dimensional scaling (MDS) was performed. To visualize the data regarding sensory attributes, differences, and similarities, scatter plots were used *(Lawless et al., 1995).*

Statistical analysis was performed firstly on the results of the following parameters: pH, TA (g/L), Brix, GSH red (ppm), GSH ox (ppm), A 280 nm, A 320 nm, A 360 nm, A 420 nm, L*, a*, b*, Cab*, hab*. Parameters were measured along the different winemaking stages: "post crushing", "post pressing", "post juice racking" and "bottling", including also the "post hyperoxygenation" stage for the treated juices. The analysis was performed using a 0,95 confidence interval and following the Kenward-Roger approach. The objective was to check any significant difference, on a statistical point of view, between the group of treated juices/wines and the group of the untreated ones. The focus of the analysis was pointed on looking for differences between stages, between treatments and finally considering the treatment*stage interaction. A separate statistical analysis was carried out on the results related to the parameters analysed via HPLC at the "bottling" stage: Tartaric acid (g/L), Malic acid (g/L), Citric acid (g/L), Succinic acid (g/L), Lactic acid (g/L), Acetic acid (g/L), Glucose (g/L), Fructose (g/L), Glycerol (g/L), Ethanol (g/L), Ethanol (%). The statistical analysis was run between all single repeats belonging to both groups of control and hyperoxygenated juices/wines and furthermore between the means of the repeats for both groups. A confidence interval of 0,95 was used and Levene's test was applied for the homogeneity of variances.

The Statistical analysis of the chemical data was performed using Statistica software version 13 (Statistica v 13, Dell Inc., Tulsa, USA). Principal Component Analysis (PCA) was performed on the scaled and centered chemical data on the HRMS results.

3.5 High Resolution Mass Spectrometry (HR-MS)

Untargeted analysis was performed on samples taken from the wines used during the sensory evaluation. The method utilised is the one described by Buica et. al (2017). Wines were analysed by UPLC (Waters Corporation) equipped with a Synapt G2 quadrupole time-of-flight mass spectrometer (Waters Corporation). The flow rate was 0.3 mL/min and the column temperature 55 ºC. The injection volume was 2 µL. The column used for separation was an Acquity UPLC HSS T3 column (1.8 µm internal diameter, 2.1 mm x 100 mm, Waters Corporation) using 0.1% formic acid (mobile phase A) and acetonitrile (mobile phase B) and a scouting gradient.

A low collision energy scan (6V) was used to acquire the data in MS^e mode. One scan from m/z 150 to 600, plus a high collision energy scan from m/z 40 to 600 with a collision energy ramp of 30-60 V. To reach an optimization for best sensitivity, the mass spectrometer was set using a cone voltage of 15 V, desolvation temperature of 275ºC and nitrogen desolvation gas at 650 L/hr. Both positive and negative mode were operated by the instrument using an electrospray ionization.

Chromatographic data was extracted as (RT_m/z, intensity) matrix by the application manager used. A MarkerLynx XS (Waters Corporation) was used to anlyse the MS data generated from both ionization

modes (separate and combined sets). This application manager it's able to integrate 3D peacks, perform data set alignment and incorporates multivariate statistical tools.

The statistical algorithms are directly applied to the processed data sets due to the direct integration of the software with SIMCA-P (Umetrics).

4 RESULTS AND DISCUSSION

4.1 Standard winemaking analysis

4.1.1 Initial level of Sugars, pH and Total Acidity

Table 4-1. Analysis of total sugars (Brix), pH and Total Acidity (measured in g/L of Tartaric Acid) after grape crushing. The results are relative to the first and the second batch of Chardonnay (C1, C2) divided into control and hyperoxygenated juices comprehensive of three repeats each (respectively CA, CB, CC and OA, OB, OC). The table shows the averages of the repeats per treatment with standard deviation. On the "Control" column, the symble "*" indicates a statistically significant difference found between treatments. The same symble colored in blu instead, indicates the difference between stages.

Batch		**C1**	**C1**	**C2**	**C2**
Treatment		**Control**	**Hyperoxygenated**	**Control**	**Hyperoxygenated**
Brix°	Average	20,57	20,37	22,07	21,77
	st.dev. +/-	0,15	0,15	0,31	0,38
pH	Average	* 3,53	3,47	3,93	3,75
	st.dev. +/-	0,00	0,00	0,00	0,00
TA (g/L)	Average	** 5,78	5,76	* 5,21	5,41
	st.dev. +/-	0,00	0,00	0,00	0,00

The results for sugars, pH, and TA are coherent with the 8 days of difference between harvests. These values confirm the higher ripening state of C2. The second batch, in fact, presents a higher sugar content, higher pH and lower acidity compared to the first batch. All parameters reside in a normal range of values, besides the pH of C2 being relatively high. However, it is not uncommon for a warm climate country like South Africa is and especially considering the area where the grapes come from. The south African regions not further away than 30-40 km from the sea are defined as 'coastal regions' and generally fall in the Winkler GDD (Growing Degree Days) Region III. Approaching more the inland instead, GDD temperature ranges fall into Region IV and somethimes V (https://www.wosa.co.za/The-Industry/Terrior/Related-Articles/Terroir-South-African-Vineyard-and-Soils-and-Climate/). Paarl is located slightly more inland than Stellenbosch meaning that its temepratures ranges from a Region III to a Region IV.

4.1.2 Fermentation curves

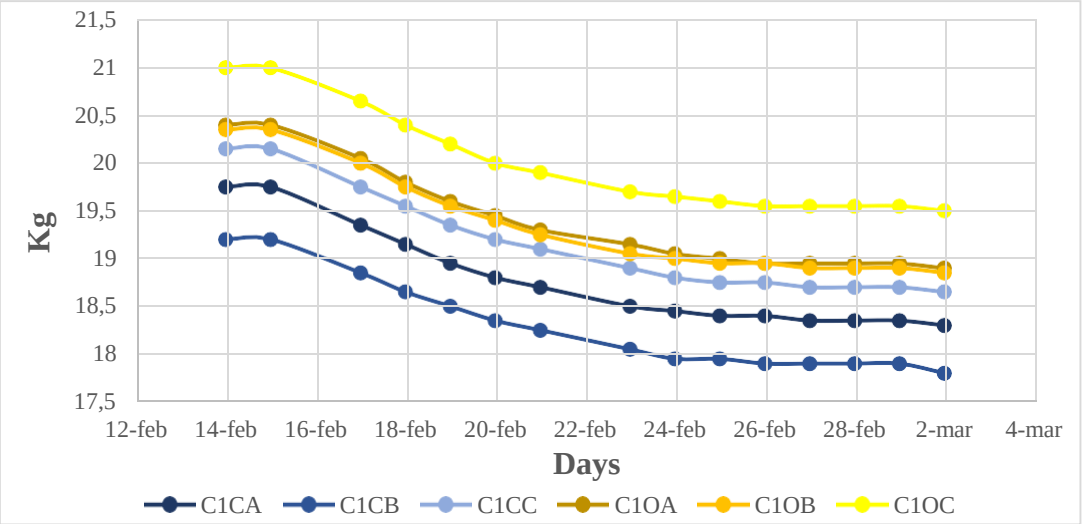


Figure 4-1. Alcoholic fermentation curve of Chardonnay batch 1, controls (CA, CB CC) and hyperoxygenated (OA, OB, OC) wines.

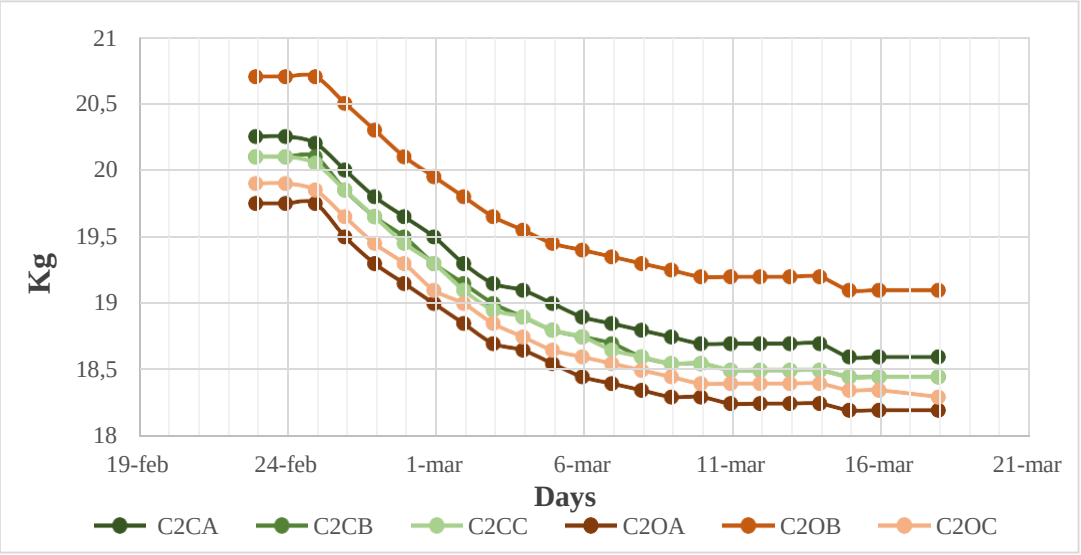


Figure 4-2. Alcoholic fermentation curve of Chardonnay batch 2, controls (CA, CB CC) and hyperoxygenated (OA, OB, OC) wines.

The alcoholic fermentation (AF) for both C1 and C2 followed a regular pattern, at 15°C room temperature. No stuck fermentation or unpredicted fermentation behaviours occured. The desired level of dryness, < 2 g/L of residual sugars, was reached on average after two weeks. No nutrients were added, considering that the initial YAN measured was higher than 150 mg/L. The decision of monitoring the fermentation by weight came from the consideration that, given the low volume of juice per batch, removed the necessity to open each time the containers to draw samples for fermentation monitoring. In

this way it was posible to avoid any further contact with oxygen after the hyperoxygenation treatment. In addition, it enabled us to evaluate only the effects related to the action of the oxygen in the precise moment when the juice was treated.

4.2 Sensory results

The effect of the hyperoxygenation treatment on Chardonnay typicality and aroma profile is presented in this section.

4.2.1 Typicality rating

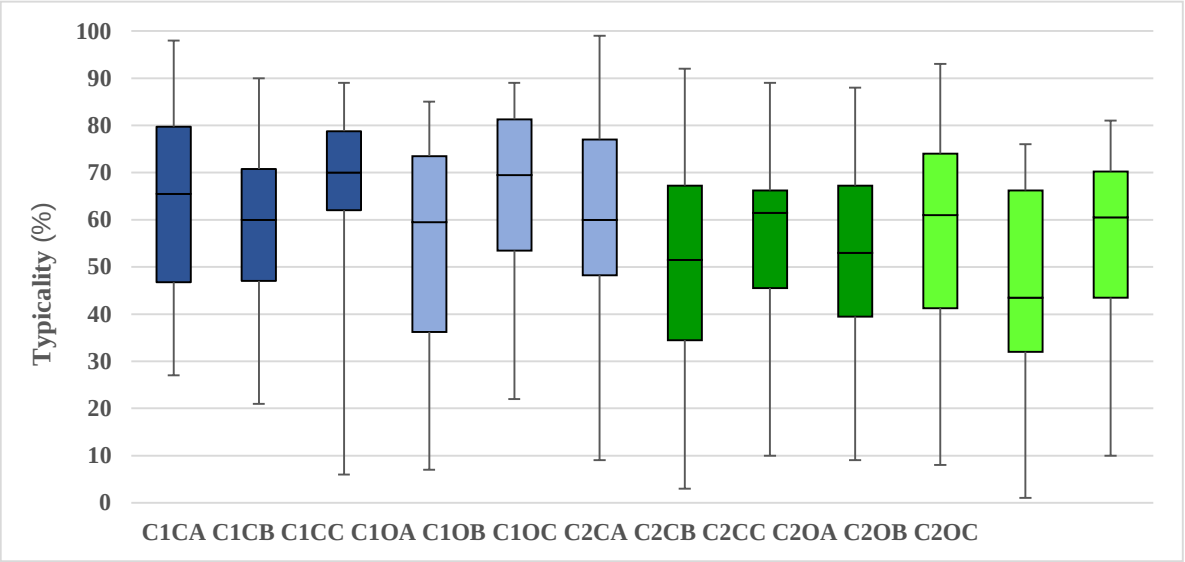


Figure 4-3. Box and Whiskers plot representing the Typcality Rating results. The sensory evaluation was performed by 20 judges on the two batches of Chardonnay (C1, C2) including both treatments (C and O) and three repeats per treatment (A, B, C).

As the boxplot shows, the values of the medians for the entire group of samples do not deviate significantly from each other. This information suggests a non-variation in typicality perception between the treated and the untreated wines. The ANOVA analysis did not show any significant difference between means. A Normality test was performed to establish the nature of the population of this experiment. Being the p-value higher than alfa (0,05) and the null hypothesis (H0) stating all means are equal, H0 cannot be rejected. This confirms the nature of the group of samples, which also do not follow a normal distribution. A non-parametric test (Kruskal Wallis) was further performed. In this case, as well, the p-value was higher than alfa (0,05). H0 cannot be rejected therefore the samples come from the same population.

The statistical results confirm what the boxplot shows: there is no variation in typicality perception among all samples, regardless of the batch and the treatment. One of the main concern of using hyperoxygenation technique is about losing the characteristic profile that makes Chardonnay recognizable by consumers. In light of this consideration, the results are positive since even wine industry experts could not pick up a perceivable typicality shift.

4.2.2 CATA – Aroma profile

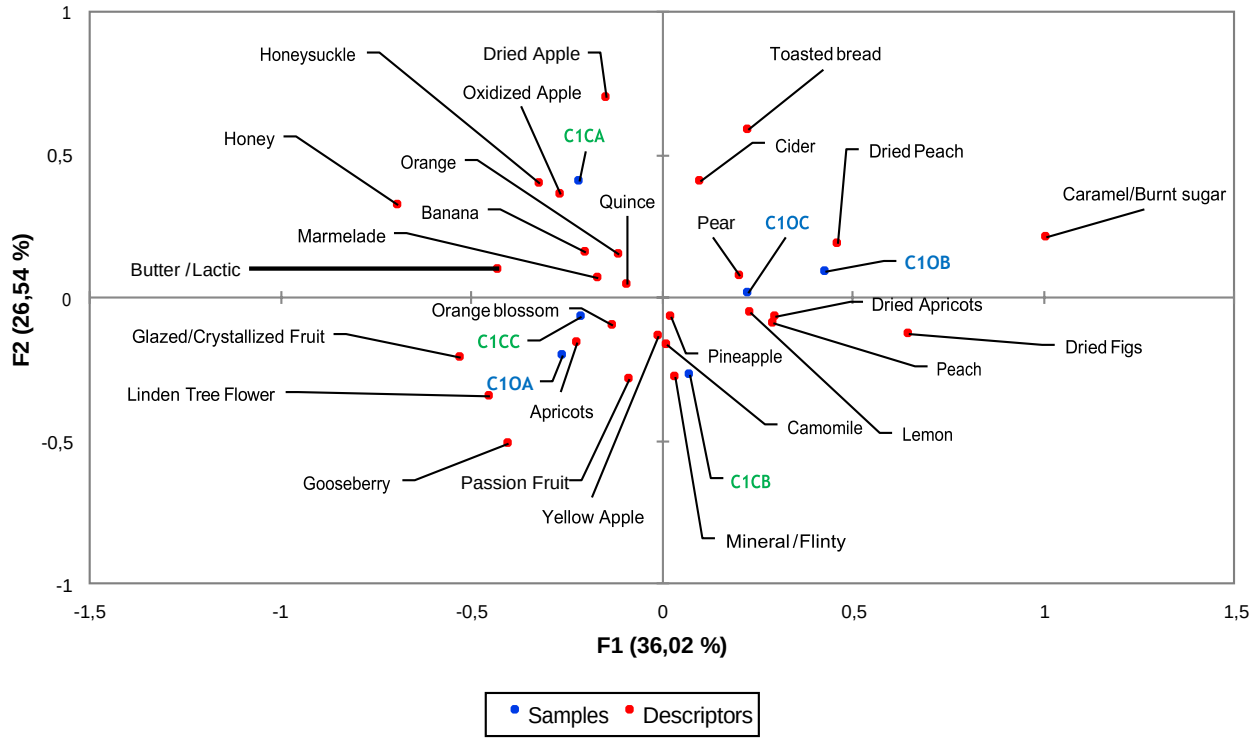


Figure 4-4. Correspondence Analysis (CA) on the wine aroma profile based on the results of the CATA evaluation for Chardonnay batch 1.

Looking at the results of the CA plot, it appears that no distinction was made between the group of control wines and the group of hyperoxygenated wines. Samples are all located relatively close to the center and in the case of C1CC and C1OA, for example, they are positioned one next to each other, despite belonging to different treatments. This data disposition, without a net separation between groups, means that there is no evident and marked effect on the aroma profile caused by the treatment. Results confirm what was found in the typicality evaluation and are indeed positives in term of maintaining the typical characteristics of Chardonnay wine, with or without using the hyperoxygenation technique.

The group of control wines of this batch shows a profile characterized by yellow fruits (‘quince’, ‘yellow apple’), tropical fruits (‘banana’,’passion fruit’, ‘pineapple’), citrus notes (’orange’, ‘lemon’), flowery aromas (‘orange blossom’), but also ‘toasted bread’ and ‘dried fruits’ aromas (‘dried apricots’). What was found to be higher in the control group was the frequency of citation for the descriptors ‘oxidized apple’ and ‘dried apples’ (especially in C1CA). ‘Orange blossom’, ‘passion fruit’ and ‘banana’ were also perceived more often in the control wines.

The group of hyperoxigenated wines shows: yellow fruits (‘quince’, ‘yellow apple’, ‘pear’ and, ‘peach’), tropical fruits (‘pineapple’, ‘passion fruit’ and ‘banana’, plus a particularly high repetition of ‘gooseberry’ in C1OA), flowery aromas (‘camomille’ and ‘orange blossom’ mainly), more citric notes than the controls, especially ‘lemon’. Aromas that were perceived the most in this group were also ‘dried fruits’ (‘dried peach’, ‘dried figs’) ‘toasted bread’, and mineral notes. Finally, the ‘caramel’ descriptor was cited more frequently in the treated wines.

On average, the frequency of citation related to the main attributes perceived for the control group is similar to the one of the treated group. Few descriptors characterize more the profile of the first type of wines: ‘orange blossom’, ‘tropical fruits’ or apple-related aromas. Other descriptors are more frequent in the second group: ‘lemon’, ‘toasted bread’, ‘dried fruits’ and ‘caramel’. These small differences are indeed present but are not so significant to divide all samples into two separate spaces, constituting a different aroma profile according to the different winemaking technique. The little differences between repeats instead, can be explained by the fact that vinification was conducted in separate canisters. This can lead to different aroma developments, even if the must come from the same pressing and the protocol followed was the same for each repeat.

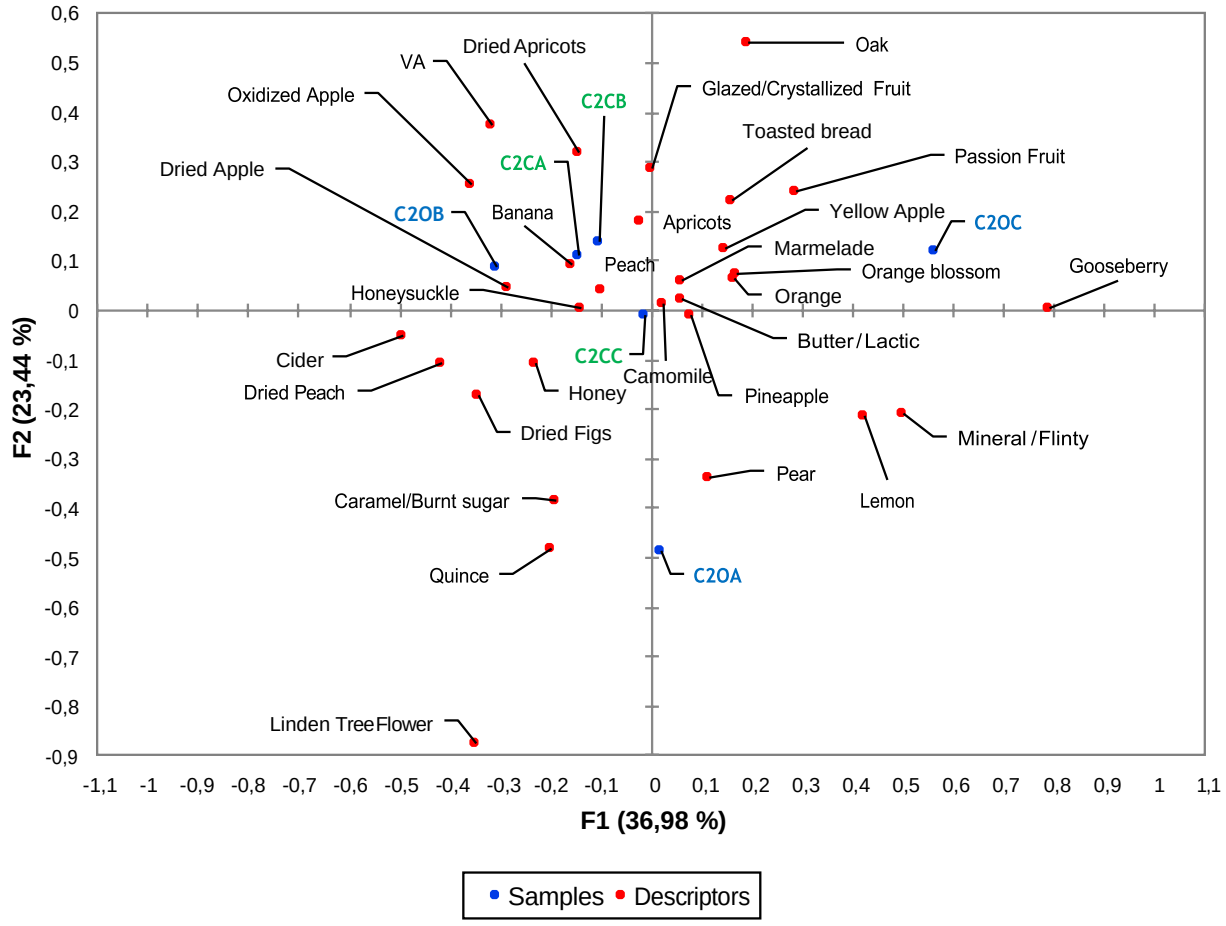


Figure 4-5. Correspondence Analysis (CA) on the wine aroma profile based on the results of the CATA evaluation for Chardonnay batch 2.

When considering the second batch of Chardonnay, we can note the following: the control samples are grouped togheter in a closed proximity, showing more consistency; the treated ones show a certain variability. This is possibly due to the hyperoxygenation process performed manually and not in a controlled manner with the aid of an oxygen meter to avoid variability within the treatment.

The control group is mainly characterized by: yellow fruits (‘yellow apple’, ‘pear’, ‘peach’), tropical fruits (‘banana’, ‘pineapple’, ‘passion fruit’), ‘oxidized apple’ and ‘citrus’ notes, flowery aromas (‘orange blossom’, ‘camomille’ and ‘honeysuckle’), ‘marmalade’ and ‘honey. In all control samples, volatile acidity (VA) was picked up by the experts. The presence of VA could be explained by the status of the second batch of grapes. They were not as healthy as the previous ones, very ripe and with a higher pH.

The treated wines had a similar profile of yellow fruits, flowery aromas, dried fruits (mainly ’dried figs’), and tropical fruits (’banana’, ‘pineapple’ and ‘gooseberry’) as the first batch of Chardonnay. The

descriptors ’lemon’, ‘toasted bread’ and ’minerality’ were more cited in this group than in the control one. Furthermore, the frequency of citations for the ‘butter/lactic’ aroma was also higher in this case. On the other side, No VA was perceived, unlike the controls. This, however, is in contrast with the data regarding the Acetic acid concentrations measured through HPLC. The results, in fact, show how the controls are the ones with lower concentrations of Acetic acid in comparison to the hyperoxygenated wines, which should present a higher VA but they were perceived having none instead.

Aromas typical of oxidation, such as ‘dried apple’ and ‘oxidized apple’, are generally perceived more in the control wines. This could be explained by accidental oxidation during the last phases of vinification or storage. The perception of these particular aromas could derive from the inferior resistance to the oxygen of the untreated wines. What can be deducted from this CATA evaluation is that control wines have a higher risk to develop possible negative oxidation aromas such as ‘oxidized apple’, even if the wines were protected as much as possible from oxygen during vinification. The hyperoxygenated wines instead, developed more ‘toasted bread’ and ‘caramel’ notes, which most likely came from the process of adding oxygen to the must after pressing. These wines also seem to be described more often as having ‘citrus’ aromas and ‘minerality’ which contributes to their freshness, despite the oxidative technique performed.

It has to be taken into account that the differences between batches are mainly related to the different must composition, which plays a very important role. To make a comparison between the two batches, we can look at the frequency of citations. The most used attributes to describe the control wines of the first batch were: ‘yellow apple’, ‘orange blossom’, ‘banana’, ‘pineapple’, ‘peach’, ‘pear’, ‘quince’ and ‘lemon’. While for the second batch: ‘yellow apple’, ‘oxidized apple’, ‘marmalade’, ‘pear’, ‘peach’, ‘pineapple’, ‘banana’, ‘lemon’, ‘dried apricots’ were chosen. Therefore, the second batch was more linked to ‘oxidation-derived’ type of aromas, probably due to its higher ripeness level.

Regarding the hyperoxygenated wines of the first batch the main descriptors were: ‘lemon’, ‘yellow apple’, ‘pear’, ‘peach’, ‘pineapple’, ‘quince’, ‘orange blossom’. For the second batch instead: ‘yellow apple’, ‘lemon’, ‘peach’, ‘pineapple’, ‘banana’, ‘quince’, ‘pear’, ‘oxidized apple’, ‘marmelade’, ‘butter/lactic’. Here again the second batch shows more oxidation related aroma plus a lactic/buttery note. The first batch on the other hand, remained more citrus, fruity and flowery.

To summarise, the first batch did not show that much ‘lactic/buttery’ aroma, such as the treated wines of the second batch. ‘Oxidized apple’ was found in both controls of the two batches but mainly in the second one. ‘Banana’ and ‘pineapple’ were higher in both controls, while ‘lemon’ was higher in both hyperoxygenated wines, confirming the findings of *Schneider (1996)* who reported an increase of the lemon aroma together with a decrease of the peach aroma after hyperoxygenation in Rieslings wines. Aromas of ‘toasted bread’, ‘dried figs’ and ‘minerality’ were also more often perceived in the treated

wines. ‘Marmelade’ was found especially in the controls of the second batch. Flowery aromas were higher for the entire first batch, ‘orange blossom’ in particular. In the second batch, fruitiness seems to increase after hyperoxygenation, confirming the results of *Cejudo-Bastante et al. (2011)* who gave as a possible explanation to a more fruity aroma, the addition of the oxygen. According to the authors, the oxygen supply can cause during AF the direction of the yeast metabolism, to shift towards the production of short- and medium-chain fatty acid esters over higher alcohols.

4.2.3 CATA - Taste and Mouthfeel

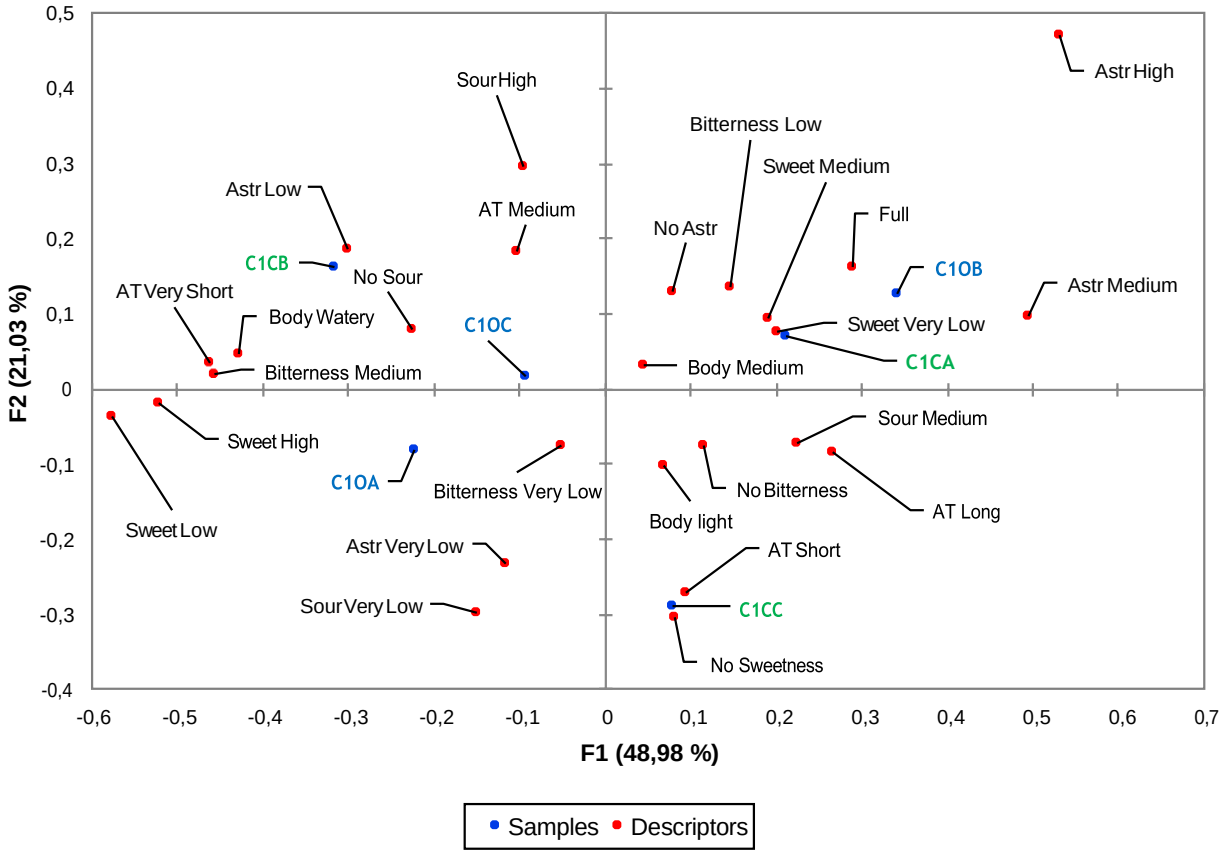


Figure 4-6. Correspondence Analysis (CA) on Taste and Mouthfeel based on the results of the CATA evaluation for Chardonnay batch 1.

Looking at the CA results, the wines belonging to both groups (control and hyperoxygenated) appears mixed again, without a net separation between groups. Therefore, hyperoxygenation did not produce significant changes in terms of taste and mouthfeel perception.

Both control and hyperoxygenated wines of the first batch shows: ‘very low sweetness’, ‘medium sourness’, ‘light-medium body’, ‘very low astringency’, ‘very low bitterness’, and ‘medium length'.

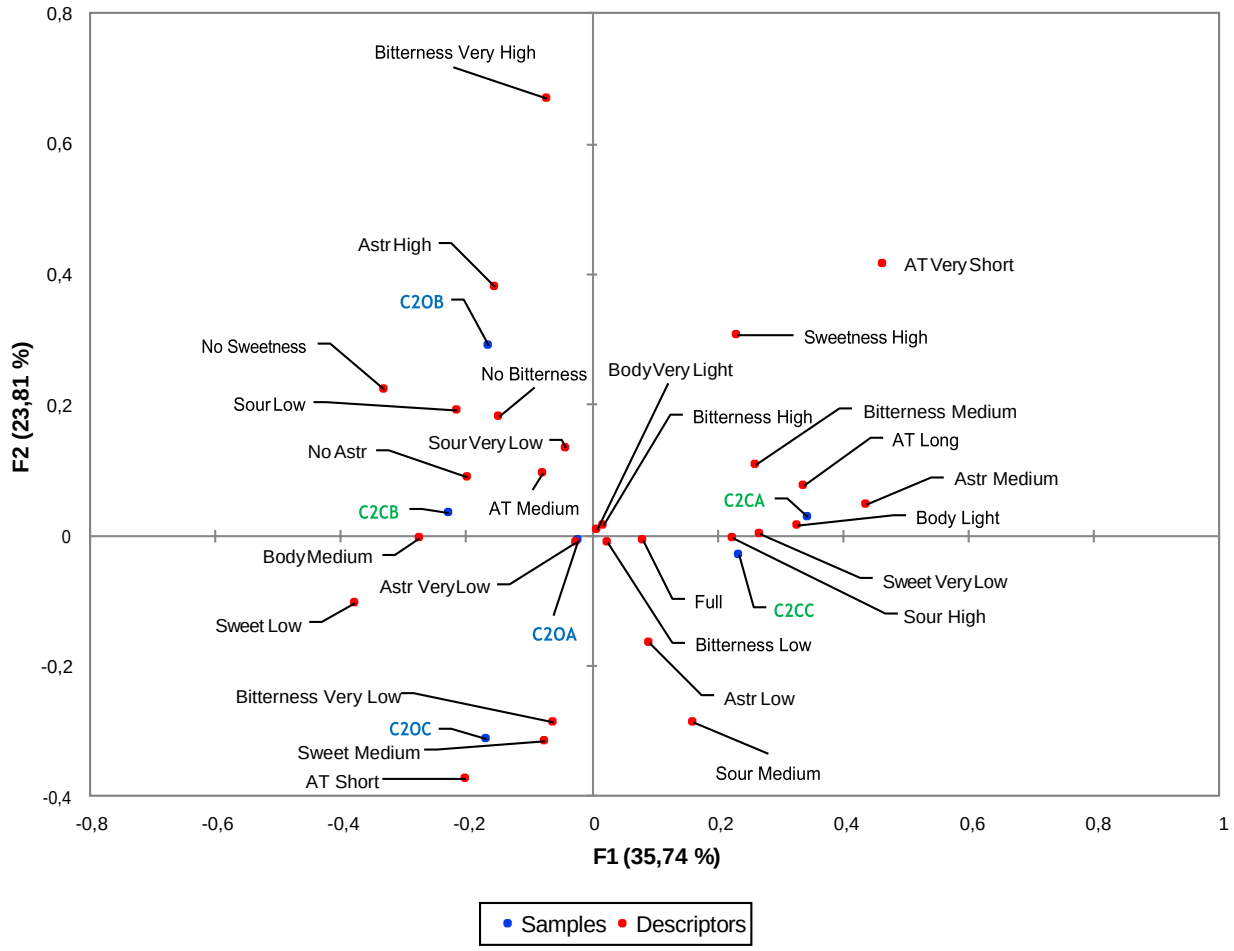


Figure 4-7. Correspondence Analysis (CA) on Taste and Mouthfeel based on the results of the CATA evaluation for Chardonnay batch 2.

When analysing the second batch results, the outcome is the same. Both control and treated wines have a very low sweetness level, 'low-medium sourness', 'light-medium body', 'very low astringency', 'very low bitterness' and 'medium length'. It is worth noticing that the profile of the first batch is similar, besides the data related to "sourness" which here is perceived to be lower than the first batch. This specific sensory result confirms the more advanced ripeness status of the grapes of the second batch (C2) and the initial chemical data taken at crushing.

Tasters were not able to identify differences between wines. Wines do not seem affected by the treatment in terms of taste and mouthfeel parameters. The level of 'astringency' and 'bitterness' was very low in both controls and hyperoxygenated wines, which is normal in white wines, but no differences were revealed between treatments. The absence of a decreased perception, in the treated wines, for 'bitterness' and/or 'astringency', could be explained by an already low initial level of phenolics in the grapes. This can derive either from the grape's composition itself or by the fact that the grapes were gently pressed

with a manual basket press. This possible explanation is supported by what stated by *Schneider (1998)* regarding the non-significant effects of hyperoxygenation on the sensory aspect in case of soft grape processing, because of the minimal release of the flavonoids in the must. Probably the phenolics that oxidized the most during the treatment, leading to such a darker color, were mainly phenolic acids. Phenolic acids, in fact, are not responsible for significant sensory aspects *(Ribéreau-Gayon et al., 2006*) and therefore they do not contribute to the final astringency and bitterness level, unlike dimers and trimers of procyanidins. The colorimetric results though, as described in the colorimetric results section, show a significant drop of both phenolic acids and flavonoids after the post hyperoxygenation racking. It means that even if both types of phenolic compounds were removed after the treatment, maybe the initial content, and therefore their precipitation, were not high enough to cause any difference in astringency and bitterness perception.

In conclusion, the typicality rating didn't show any significant difference which means that, even if the wines were perceived to be different from each other and having not exactly the same principal descriptors, this still didn't affect the typicality and the recognizability of the grape variety by the tasters. The results are in disagreement with *Singleton et al. (1980),* who stated that hyperoxygenation decreases excessively the aromatic and phenolic profile of the wine provoking, not only lack of aromas intensity, but also a loss of cultivar typicality.

From the CATA results, it seems that the hyperoxygenation treatment could affect the wine on a stylistic point of view, giving a product with slightly different aroma characteristics but without varying too much from the results obtained by conventional winemaking. The results are in agreement with the findings of *Cheynier et al. (1991*), who reported no aroma or quality degradation in the wines after hyperoxygenation. Aromatic properties were also not significantly affected by the treatment, according to the author. The small changes in the aroma composition, not perceived as negative, were also found by *Cejudo-Bastante et al. (2011).* The latter author though, noticed a decrease of the flowery notes in the treated wines, together with an increase of freshness and 'banana' aroma. In this experiment instead, flowery aromas were perceived more frequently in the hyperoxygenated wines, while banana aromas were less often perceived in comparison to the controls. Regarding freshness, the treated wines here showed more often citrus ('lemon') aromas and minerality which could be linked to 'freshness'.

Hyperoxygenation might not be the ideal option for a producer who is prioritizing the maintenance of a certain wine style to the introduction of a different production step and its benefits. On the contrary, this could definitely be a valid option for producing a wine with a reduced sulfur dioxide content. It is also a valid alternative for press juice, which tends to have a high concentration of phenolic compounds. Phenolics could then be reduced through hyperoxygenation, making the wine lighter and easier to drink, while reducing the risk of further oxidation and the darkening of the color during storage. Letting the

polyphenols oxidize and avoid the use of nitrogen or another inert gas like CO_2 during the first stages of vinification, is a money-saving practice especially in case of large productions. At the must stage, the oxidation risk is higher due to the PPO activity, therefore, a consistent amount of inert gas is used to avoid both browning and loss of aromas.

In case of a classic method of sparkling wine production, hyperoxygenation could be a valid technique for the preparation of the base wines to avoid future color instabilities and reduce the SO_2 content. In fact, for this type of product, the main aromas come from the yeast activity during the second fermentation so the primary aromas that could potentially be affected by the treatment, play a less important role

4.3 Chemical results

4.3.1 HPLC - Sugars, Organic Acids, and Ethanol content

HPLC analysis was performed to measure the concentration of Sugars, Organic Acids and Ethanol content at the bottling stage. Between treated and untreated wines from batch 1 and 2, no significant difference was revealed. The results are not surprising considering that neither oxygen or hyperoxygenation should affect the concentration of these parameters. Data are reported in the following tables.

Table 4-2. HPLC results on Organic Acids concentration for Chardonnay batch 1 (C1) and batch 2 (C2); controls (C1C and C2C) and hyperoxygenated wines (C1O, C2O). Three repeats (A, B, C) were analyzed for each treatment and batch of wines. Data are expressed in g/L. The table reports the results of the averages of the repeats per treatment with standard deviation. On the "Control" column, the symble "*" indicates a statistically significant difference found between treatments.

Batch		**C1**	**C1**	**C2**	**C2**
Treatment		**Control**	**Hyperoxygenated**	**Control**	**Hyperoxygenated**
Tartaric Acid	Average	2,76	2,77	2,34	2,41
	st. dev. +/-	0,04	0,04	0,06	0,12
Malic Acid	Average	3,13	3,01	* 4,14	4,41
	St. Dev.	0,03	0,02	0,04	0,23
Citric Acid	Average	0,39	0,37	0,48	0,46
	St. Dev.	0,00	0,00	0,00	0,01
Succinic Acid	Average	1,53	1,47	2,30	1,85
	St. Dev.	0,03	0,11	0,08	0,07
Lactic Acid	Average	0,22	0,20	0,22	0,21
	St. Dev.	0,01	0,02	0,03	0,03
Acetic Acid	Average	0,13	0,20	* 0,17	0,46
	St. Dev.	0,12	0,02	0,01	0,25

Table 4-3. HPLC results on Sugars concentration for Chardonnay batch 1 (C1) and batch 2 (C2); controls (C1C and C2C) and hyperoxygenated wines (C1O, C2O). Three repeats (A, B, C) were analyzed for each treatment and batch of wines. The table reports the results of the averages of the repeats per treatment with standard deviation. On the "Control" column, the symble "*" indicates a statistically significant difference found between treatments.

Batch		**C1**	**C1**	**C2**	**C2**
Treatment		**Control**	**Hyperoxygenated**	**Control**	**Hyperoxygenated**
Sucrose g/L	Average	1,30	1,32	0,79	1,43
	st.dev. +/-	0,02	0,03	0,01	0,58
Glucose g/L	Average	0,86	0,89	* 0,91	0,55
	st.dev. +/-	0,03	0,01	0,05	0,26
Fructose g/L	Average	0,58	0,60	* 0,62	0,46
	st.dev. +/-	0,02	0,03	0,03	0,12

Table 4-4. HPLC results on Glycerol and Ethanol concentration for Chardonnay batch 1 (C1) and batch 2 (C2); controls (C1C and C2C) and hyperoxygenated wines (C1O, C2O). Three repeats (A, B, C) were analyzed for each treatment and batch of wines. The table reports the results of the averages of the repeats per treatment with standard deviation.

Batch		**C1**	**C1**	**C2**	**C2**
Treatment		**Control**	**Hyperoxygenated**	**Control**	**Hyperoxygenated**
Glycerol g/L	Average	0,30	0,29	0,30	0,29
	st.dev. +/-	0,01	0,02	0,03	0,03
Ethanol g/L	Average	92,98	93,02	101,28	100,99
	st.dev. +/-	1,06	0,50	0,94	1,57
Ethanol %	Average	11,78	11,79	12,84	12,80
	st.dev. +/-	0,13	0,06	0,12	0,20

4.3.2 Glutathione

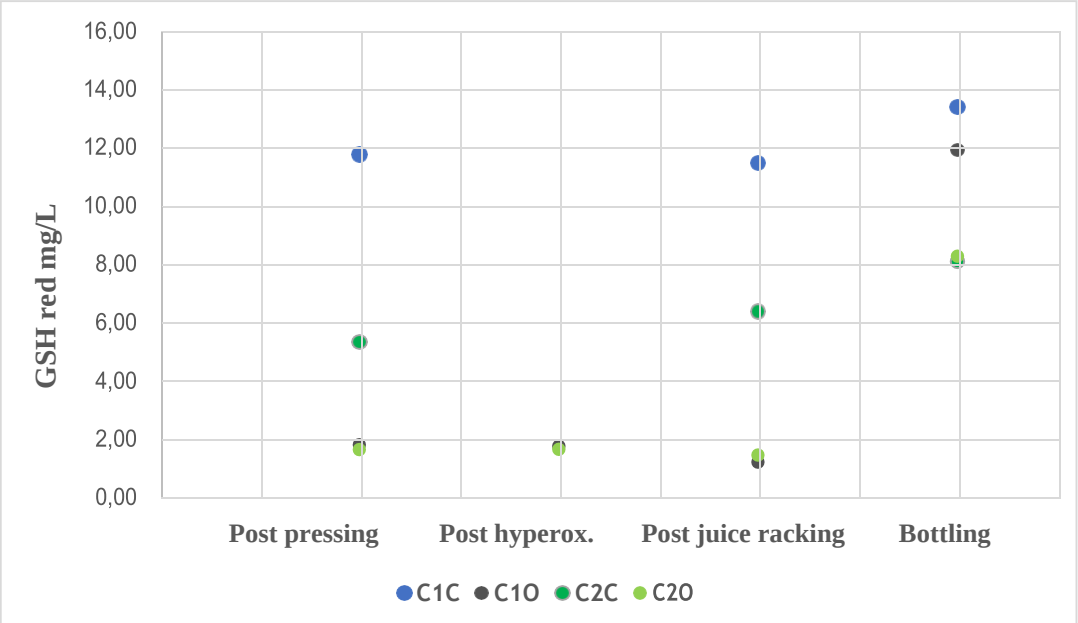


Figure 4-8. Concentration in mg/L of reduced glutathione in treated (C1O, C2O) and untreated (C1C, C2C) must/wines from the two batches of Chardonnay (C1, C2). Samples were analyzed at different stages: "post pressing", "post hyperoxygenation", "post juice racking" and at "bottling".

The data in Fig. 4-8. shows how, from the pressing stage, a decrease in glutathione content appears evident for both groups of treated wines (C1O, C2O) in comparison with their controls (C1C, C2C). Between batches, C2 has a significant lower content of glutathione. This is due to the higher maturity level of this batch, especially in terms of pH. As stated by *Webber et al. (2014)*, the low pH of the wine promotes the reduced form of glutathione. The initial results on must pH show in fact, a value of 3.53 for the controls of the first batch and 3.93 for the controls of the second batch.

After crushing no sulfur dioxide was added in the musts meant to be hyperoxygenated, therefore the oxidation process had already begun before the treatment itself, causing a decrease in the concentration of reduced glutathione which was acting as an antioxidant agent. The content of the GSH in its reduced form was, in fact, inferior in C1O and C2O, because of the combination of glutathione with *o*-quinones, to form the Grape Reaction Product, as explained by *Kritzinger et al. (2012)*. The data is in line with what *Li et al. (2008)* reported about finished hyperoxigenated wines, which contained not only a lower amount of polyphenols, but also higher concentrations of Grape Reaction Product.

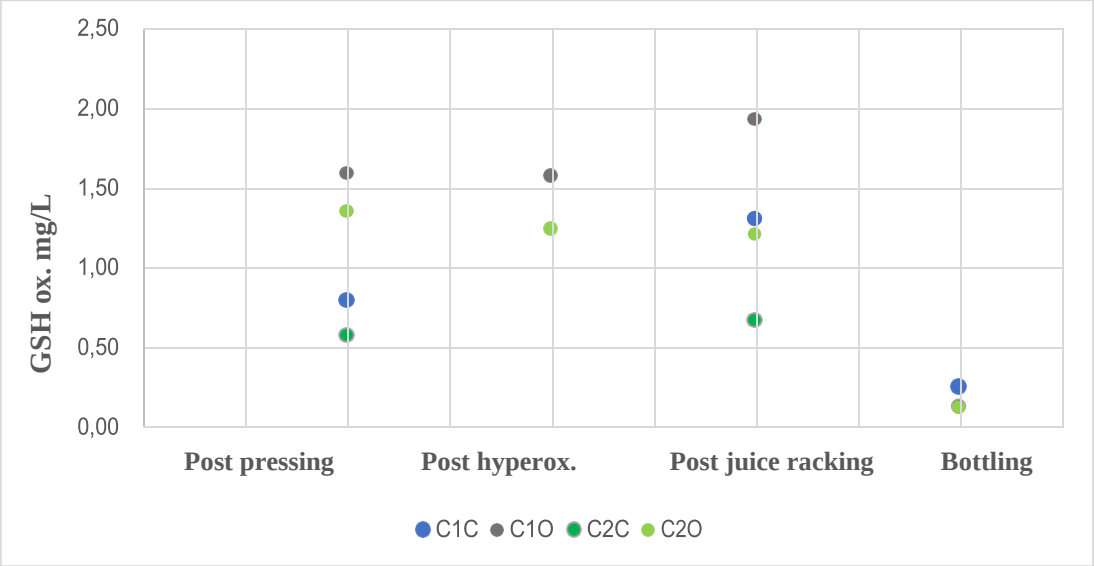


Figure 4-9. Concentration in mg/L of oxidized glutathione in treated (C1O, C2O) and untreated (C1C, C2C) must/wines from the two batches of Chardonnay (C1, C2). Samples were analyzed at different stages: "post pressing", "post hyperoxygenation", "post juice racking" and at "bottling".

On the contrary, C1O and C2O present higher values for the oxidized form of GSH in comparison to their controls (C1C, C2C). The glutathione in fact already played its protective role against oxygen. Most of it is found here in its oxidized form which, as stated by *Cheynier et al. (1989)* it is not readily available to trap the *o*-quinones.

It makes sense that the depletion of GSH happens at a much faster rate when the must undergoes a high exposition to oxygen. The grapes with a lower initial content of GSH and higher pH might show a browning effect quicker than others. The low GSH concentration might influence the results concerning color browning but, of course, does not explain those results by itself. Nevertheless, it is one of the variables which can contribute to the changes of the must's color which will be discussed more in details in the following sections.

Theoretically, to perform hyperoxygenation, it might be easier to see the effects on phenolic precipitation with grapes which starts already with a low GSH concentration. On the other hand, being the must of those varieties more susceptible to the oxidative phenomena, control wines might undergo as well partial and unwanted oxidation, if not protected from the action of the oxygen. This can be seen with the colorimetric data in the next section where, contrarily to the first batch, not only the hyperoxygenated wines but also the controls in C2, shows a decrease of their absorbances. This indicates a decrease in phenolic compounds concentration. Even if inferior, the decrease was still noticeable in the wines protected from oxidation. Once more, glutathione content is not the only explanation to phenolic susceptibility to oxygen but it can contribute to explaining the behavior of this second batch.

A batch with a lower initial content of GSH, higher pH and maturity level, and a higher loss of polyphenols even in the control wines.

4.3.3 Colorimetric measurements - UV-Vis

Absorbances were measured by UV-Vis at four different wavelengths: 280 nm for total phenolics, 320 nm for phenolic acids, 360 nm for flavonoids and 420 nm for the yellow pigment.

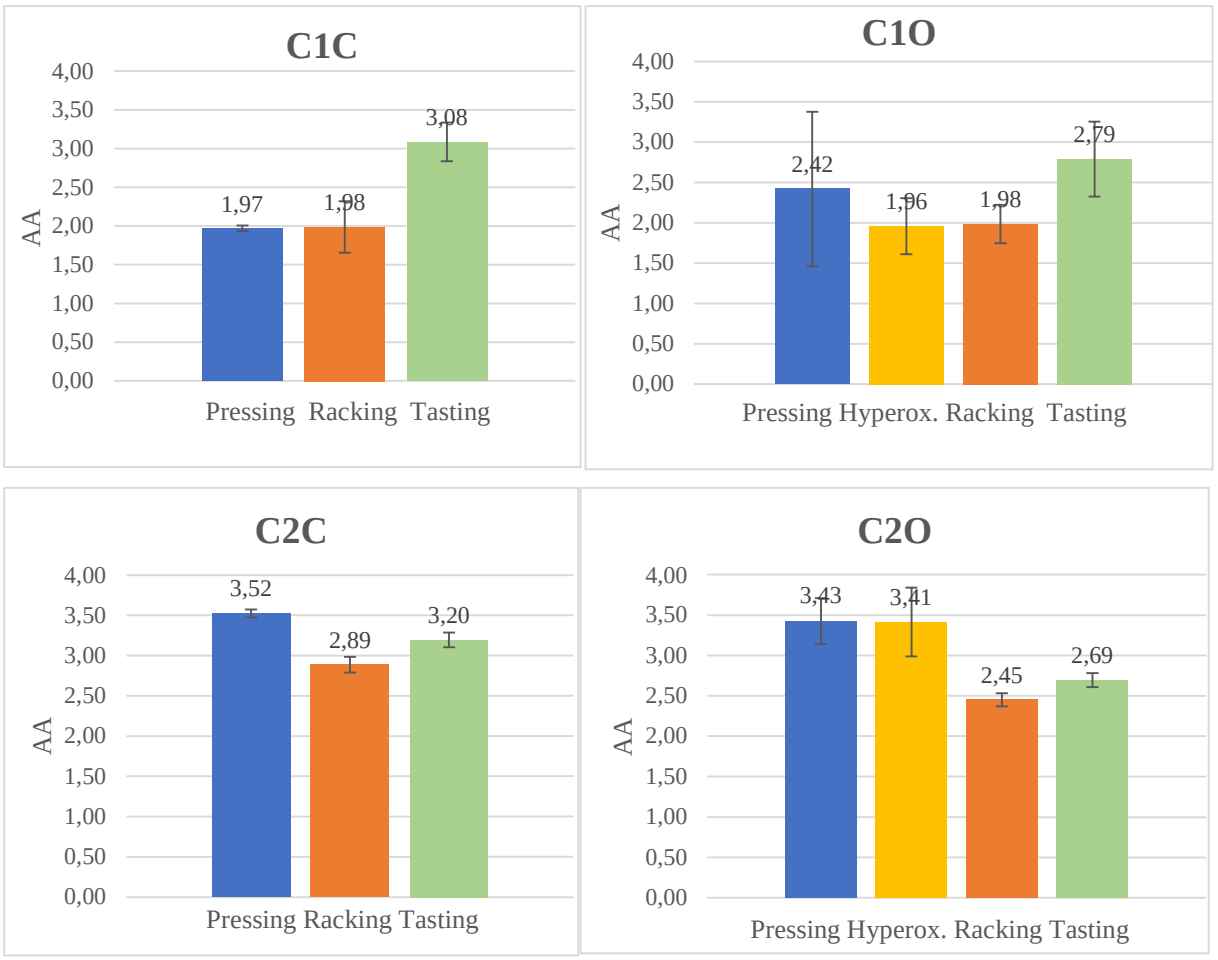


Figure 4-10. UV-Vis Absorbances measured at 280 nm (total phenolics) for Chardonnay batch number 1: Control juice/wine (C1C) and hyperoxygenated (C1O) and for Chardonnay batch number 2: Control (C2C) and hyperoxygenated (C2O). The table shows, for both batches and treatments, the average of the three repeats with the standard deviation according to each stage.

The graphs show the evolution of the absorbance at 280 nm. The measurement at this specific length indicates the concentration of total phenolics in the juice or the wine. Total phenolics in C1C seems to increase along the process, from “post pressing” until “tasting”, while they first decrease and then increase in the treated wines (C1O). The general decrease of the absorbance at 280 nm was also reported

by *Ricardo -da -Silva et al. (1993)* as a consequence of browning. It makes sense that the total phenolic content drops with hyperoxygenation, due to the precipitation after phenolic oxidation and polymerization. It is evident during the second and third stage here represented, respectively "post hyperoxygenation" and "post juice racking". The increase at the tasting stage can be explained by the formation of new phenolic compounds during the elapsed time from fermentation to the storage, until the day of the tasting. It has to be taken into account, that phenolics are not static compounds, but they evolve and change their structure during vinification and aging. Therefore, it is possible that they were reformed again, even if a consistent amount already precipitated before.

On the other side, the controls of the second batch (C2C) undergo a general drop in concentration from the beginning until the end, having a similar behavior of the respective oxidized wines (C2O), probably because they were subjected to accidental oxidation. This batch of grapes was more ripe and contained less glutathione than the first one. This two factors might have influenced the total phenolic concentration results. For the hyperoxygenated juices/wines, the absorbance drop is not reported after the hyperoxygenation stage as expected. This can be due to a sampling error or perhaps the precipitation process was simply slower in this case, considering that the concentration decreases anyway after "juice racking". The final content of total phenolics in C1O and C2O though remains inferior in comparison to the respective control wines (C1C, C2C) which means that part of the phenolics was indeed removed with the treatment, as confirmed by the HRMS analysis. These results confirm what was found in the literature by *Li et at. (2008)* concerning the lower amount of polyphenols that should be present in finished wines compared to the respective controls that didn't undergo hyperoxygenation. The latter ones, according to the author, will have a higher polyphenol content and therefore a higher browning potential.

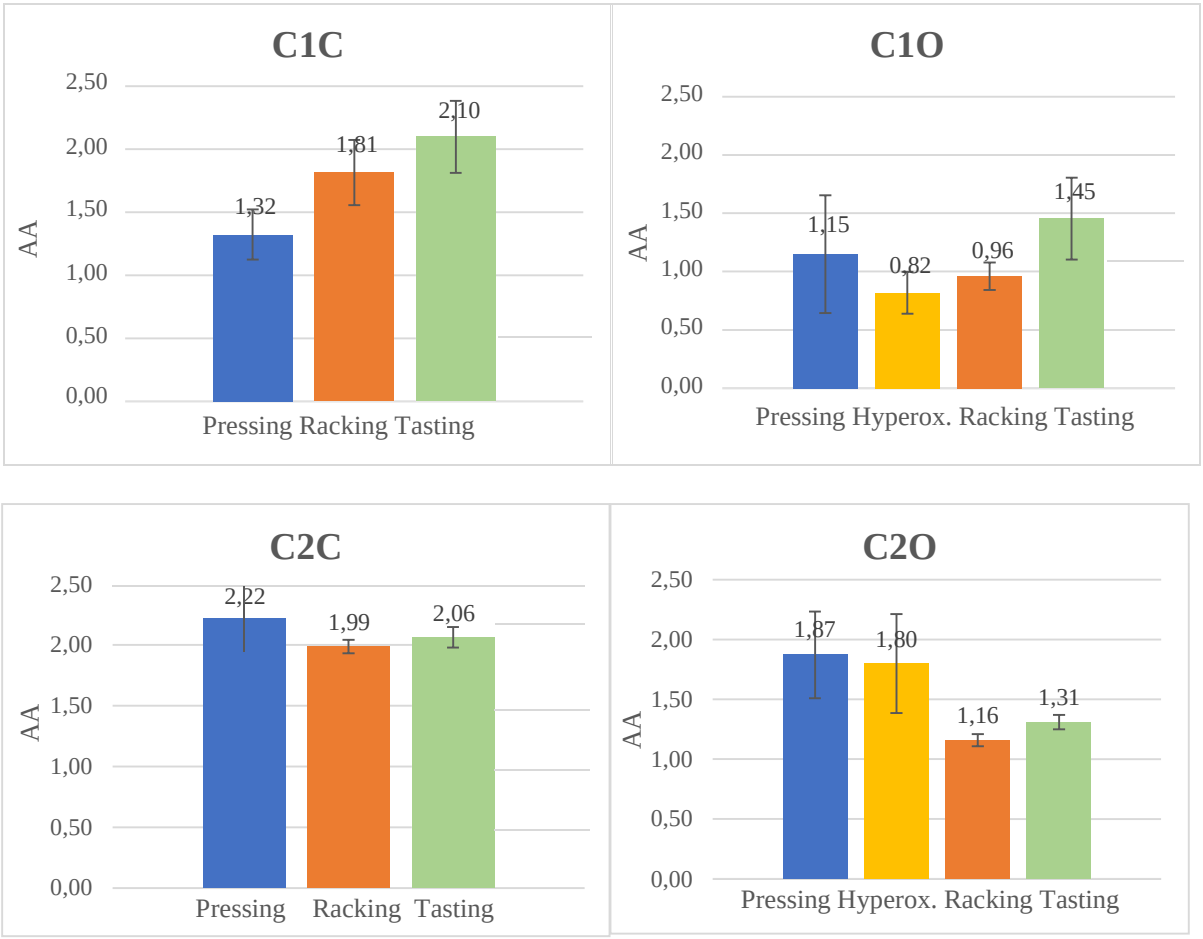


Figure 4-11. UV-Vis Absorbances measured at 320 nm (phenolic acids) for Chardonnay batch number 1: Control juice/wine (C1C) and hyperoxygenated (C1O) and for Chardonnay batch number 2: Control (C2C) and hyperoxygenated (C2O). The table shows, for both batches and treatments, the average of the three repeats with the standard deviation according to each stage.

The Absorbance at 320 nm indicates the phenolic acids content. In this case, the absorbance for the C1C group keeps increasing while the C1O first drops because of phenolic acids precipitation and finally raises again, showing the same behavior noticed for the 280 absorbances. The C2C group instead, remains more or less invariant for the control and decrease for the hyperoxygenated, but only after juice racking, showing again that the precipitation was probably slower in this case. The second batch appears to be more affected by the treatment which means that the initial concentration of phenolic acids in the must composition of C2 was higher than in C1, as can be seen from the initial AA of both control groups. In both batches, the effects of the treatment appear evident. The results confirm the decreasing concentration of phenolic acids, due to hyperoxygenation, described by *Cheynier et al. (1991)*. The differences between the two batches depend on the different must composition and the evolution of phenolics until the tasting time.

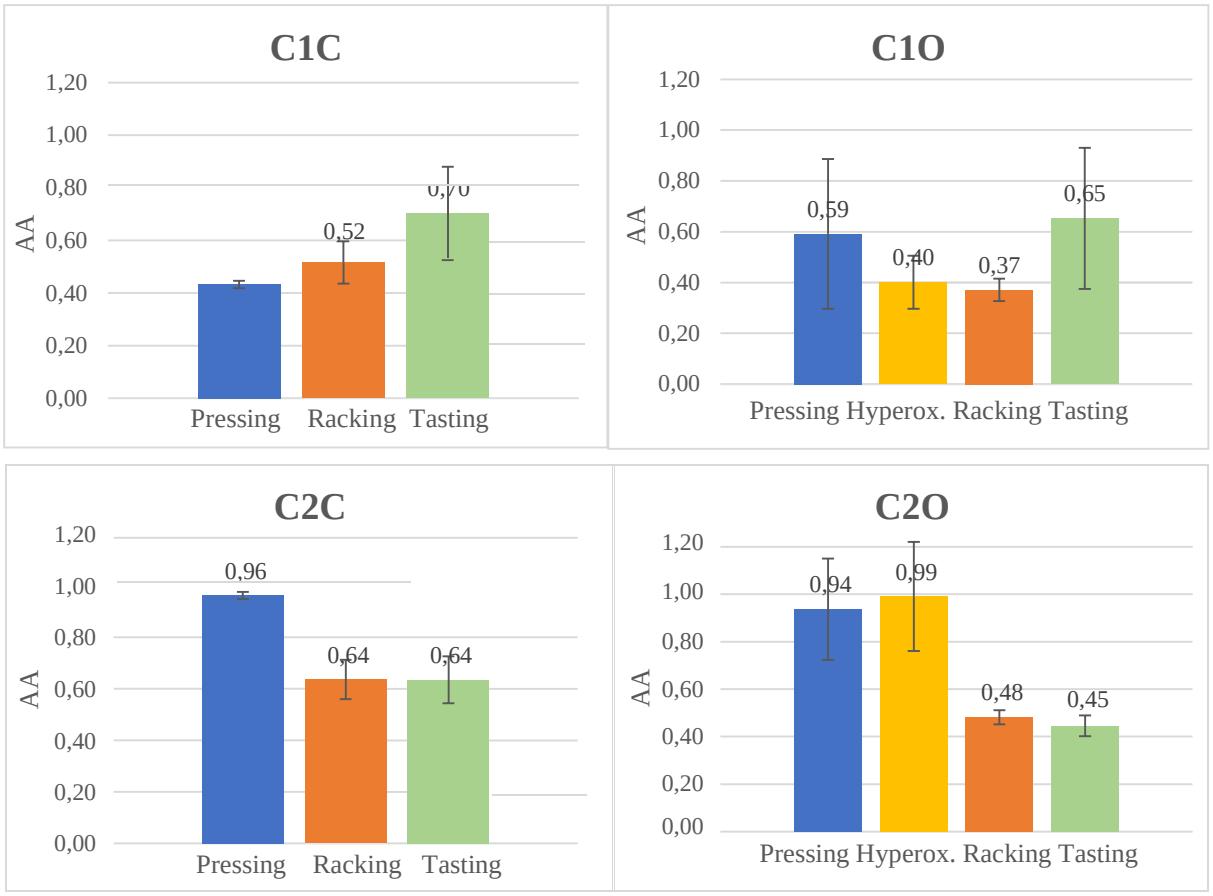


Figure 4-12. UV-Vis Absorbances measured at 360 nm (flavonoids) for Chardonnay batch number 1: Control juice/wine (C1C) and hyperoxygenated (C1O) and for Chardonnay batch number 2: Control (C2C) and hyperoxygenated (C2O). The table shows, for both batches and treatments, the average of the three repeats with the standard deviation according to each stage.

In C1C there is a slight increase in the concentration of flavonoids while for C1O it drops and then raises again, as it was seen with the previous absorbances at 280 nm and 320nm. Whereas for the second batch, the control group (C2C) and even more the hyperoxigenated one (C2O) significantly decrease their concentration in flavonoids. The initial flavonoids content in the must be higher in C2 than in C1, as seen for total phenolics and phenolic acids. Looking at the absorbances values for C2 at "post pressing" stage, in fact, it is clear how much higher the concentration of flavonoids is compared to the first batch. It seems that higher is the initial phenolic concentration, higher is the effect of the hyperoxygenation treatment. This lead to the conclusion that this winemaking technique would be very effective in the case of press juice, where the phenolic content is much higher than the free-run juice.

However, the controls (C2C) show a significant decrease too. Considering that the drop in total phenolics was not intense, the reason for this behavior is most likely the polymerization and formation

of other phenolic compounds. Another option could take into account the high sensitivity of flavonoids to the action of oxygen. In the latter case, if the must went under accidental oxidation during storage, that could have caused precipitation and a decrease of the flavonoids content.

Overall we can say that the general behavior for C1C follows an overall increase of the absorbances (280 nm, 320 nm and 360 nm) along the process while for C1O there is firstly a reduction at the "post hyperoxygenation" and "post racking" stages, followed by an increase at the "tasting" stage, possibly due to a reformation of the compounds during vinification and storage. The second batch instead, doesn't show any decrease after the "post hyperoxygenation" stage but it does in the "post racking" one. This can be due to a sampling error or to a slower polymerization process. However, both controls (C2C) and treated wines (C2O) have an overall decrease of the absorbances (280 nm, 320 nm and 360 nm) from the beginning until the end. Furthermore, in the second batch, flavonoids and phenolic acids are more affected by the oxygen action and show a higher decrease than the first batch. After all, the total phenolic content was also higher in C2 meaning that the effects of the treatment were more evident in this case.

After racking, the content of flavonoids in the hyperoxygenated wines significantly decreases, proving what stated by *Ricardo da Silva et al. (1993)* about the large removal of these precursors of tannins after hyperoxygenation. At the tasting stage though, the concentration of flavonoids raised again reaching a value similar to the controls. Perhaps this could be the explanation to the absence of variability perceived on bitterness and astringency by the tasters during the CATA sensory evaluation. Also, the flavonoids threshold decrease when their polymerization degree increase, therefore a higher polymerization degree could explain the lack of differences in astringency and bitterness.

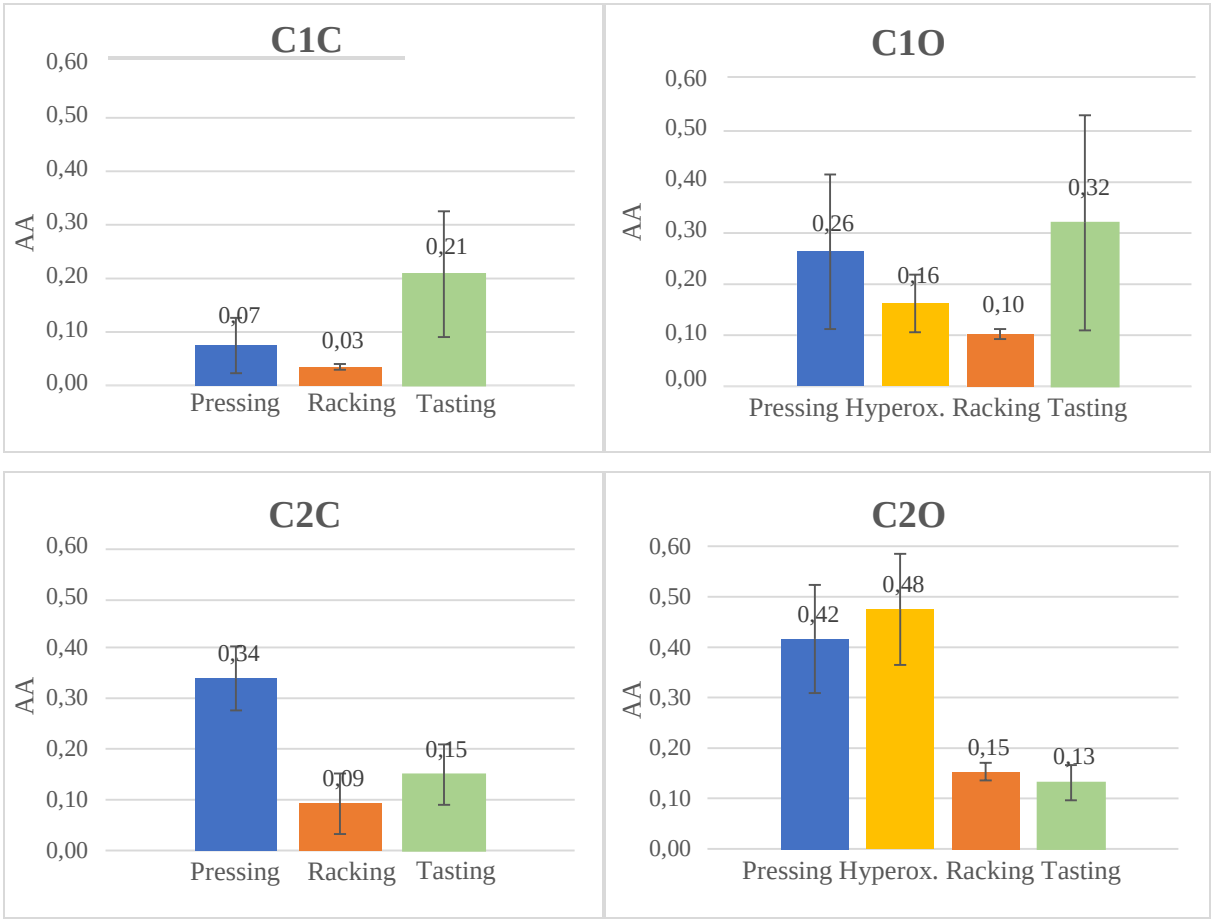


Figure 4-13. UV-Vis Absorbances measured at 420 nm (yellow pigmentation) for Chardonnay batch number 1: Control juice/wine (C1C) and hyperoxygenated (C1O) and for Chardonnay batch number 2: Control (C2C) and hyperoxygenated (C2O). The table shows, for both batches and treatments, the average of the three repeats with the standard deviation according to each stage.

At the 'post pressing' stage, the yellow pigment, an indicator of browning, is much higher in the second batch compared to the first one, probably because of the higher content in C2 of total phenolics and especially flavonoids. The initial yellow pigment is particularly high in the hyperoxigenated juices too (C1O, C2O), because the browning already started during pressing, in the absence of sulfur dioxide. For the controls (C1C, C2C) the absorbance value raises after juice racking confirming what stated by *Ricardo-da-Silva et al. (1993)* about the increased absorbance at 420 nm after clarification, as a consequence of the browning effect. The browning effect, in fact, should be more evident in wines with higher polyphenols content, therefore the controls, as confirmed by *Li et at. (2008).*

The values of the control group in the first batch (C1C) shows a must that increases its susceptibility to browning during time, having in the end higher values for the yellow pigment, as was expected. The result is in according to *Li et at. (2008)* who stated that the non-hyperoxygenated wines show a higher browing potential. The treated group (C1O) though, firstly see a decrease in yellow pigmentation once

precipitation happens, but then it increases again, even more than the respective controls and unlike what happens with the treated wines of the second batch (C2O). Probably not all the compounds responsible from browning were removed, or they got reformed in a second moment.

On the other side, the proportional decrease of the yellow pigment in C2 it is much more intense for both groups. The second batch was the one showing higher content of flavonoids which are the main indicators of browning susceptibility (*Cheynier et al., 1989*). This explains the higher absorbances (420 nm) for yellow pigmentation in C2 for the first two stages and the inferior values for the last two stages, when the flavonoids content significantly dropped due to the hyperoxygenation.

It seems that both C2C and C2O absorbances dropped along the process from "post juice racking" and then kept the lower level in the end. The values for the yellow pigment were initially higher in C2 than C1 because of the higher total phenolic content of this batch. Therefore, the compounds responsible for browing were initially higher in this must. After phenolic precipitation though, the yellow pigmentation decreased meaning that the compounds responsible for that were removed. The fact that the absorbance at 420 nm decreases for the controls as well (C2C), even if was not expected, it makes sense considering the decrease of all absorbances previously measured (280 nm, 320 nm, 360 nm) for this group and at these stages. It seems that partially the phenolics were removed in the control (C2C) as well, probably because of a higher sensitivity to oxygen for this must composition.

Table 4-5. Percentage of overall absorbance's increase from "post pressing" until "tasting". The values were calculated considering the average of the three repeats (A, B, C) for each group of wines: treated and untreated (O, C). On the left Chardonnay batch 1 and the right Chardonnay batch 2.

C1	Control	Hyperoxygenated
280 nm	56%	15%
320 nm	58%	26%
360 nm	62%	10%
420 nm	177%	21%

C2	Control	Hyperoxygenated
280 nm	-9%	-21%
320 nm	-7%	-30%
360 nm	-34%	-52%
420 nm	-56%	-68%

Table 4-5. shows in percentage how all the absorbances overall increase or decrease for all groups of wines. In C1 the decrease percentage is much higher in the treated wines compared to the controls. It means that the treatment did reduce the concentration of total phenolics, phenolic acids, flavonoids, and yellow pigment. The fact that those compounds decreased in the controls as well it is possibly due to the precipitation that all wines undergo when submitted to clarification, even more, when using a pectolytic enzyme.

On the opposite, C2 shows an overall increase of all absorbances but the increase of the hyperoxygenated is much lower than one of the control wines meaning that the treatment did have an effect here as well.

Looking at the absorbance at 420 nm, it seems that the controls increase the yellow pigment of 177% from pressing to tasting, while the hyperoxygenated only 21 %, showing more resistance to browning.

In conclusion, the percentages' sign for absorbance increase is negative in C1 and positive in C2. The wines show an opposite behavior in terms of phenolic evolution and this is related to the must composition and the phenolic changes and evolution during vinification and wine storage. However, the behavior in regards to hyperoxygenation was the same for both batches, showing in both cases success in the removal of phenolic compounds and a reduction in browning susceptibility.

4.3.4 Cie-Lab results

CieLab parameters were calculated from the absorbances values measured with the UV-Vis.

Table 4-6. Calculation of the CieLab parameters in Chardonnay batch 1. The data represents the average of the three repeats (A, B, C) per each treatment (C, O) plus their standard deviation. The symble "*" in blu indicates, for that parameter, a significant statistical difference found between stages. The same symble "*" in red, between treatments.

C1	Post pressing		Post hyperoxygenation		Post juice racking		Tasting	
	average	st.dev. +/-	average	st.dev. +/-	average	st.dev. +/-	average	st.dev. +/-
L* C1C *	97,40	2,16			99,36	0,09	89,07	6,75
L* C1O *	91,18	5,09	94,44	2,19	97,17	0,14	82,96	11,34
a* C1C *	-0,89	0,27			-0,95	0,15	-1,36	0,35
a* C1O *	-2,29	0,94	-1,84	0,36	-1,55	0,16	-1,45	0,35
b* C1C	4,14	2,73			2,19	0,35	6,56	2,77
b* C1O	13,46	7,03	8,76	2,81	6,16	0,70	8,31	4,10
Cab* C1C	4,25	2,71			2,39	0,38	6,70	2,78
Cab* C1O	13,65	7,08	8,95	2,82	6,36	0,72	8,45	4,09
hab*C1C *	-75,19	7,48			-66,45	0,54	-77,51	2,81
hab*C1O *	-79,70	2,04	-77,70	1,92	-75,83	0,19	-79,00	3,46

Table 4-7. Calculation of the CieLab parameters in Chardonnay batch 1. The data represents the average of the three repeats (A, B, C) per each treatment (C, O) plus their standard deviation. The symble "*" indicates, for that parameter, a significant statistical difference found between stages.

C2	Post pressing		Post hyperoxygenation		Post juice racking		Tasting	
	average	st.dev. +/-	average	st.dev. +/-	average	st.dev. +/-	average	st.dev. +/-
L* C2C *	87,72	1,79			97,62	2,35	92,99	3,34
L* C2O *	85,37	3,81	82,49	3,87	95,62	0,48	94,04	1,98
a* C2C	-2,07	0,53			-1,23	0,62	-1,38	0,24
a* C2O	1,73	0,26	-2,06	0,11	-1,78	0,15	-1,45	0,17
b* C2C *	16,00	3,60			5,73	3,65	5,75	1,50
b* C2O *	19,45	3,87	20,78	3,55	8,85	0,95	5,25	0,95
Cab*C2C *	16,16	3,48			5,95	3,47	5,91	1,51
Cab*C2O *	19,56	3,84	20,78	3,52	9,03	0,96	5,45	0,96
hab* C2C	-82,04	4,08			-73,20	11,96	-76,33	1,40
hab* C2O	-84,05	1,63	-84,17	1,28	-78,64	0,24	-74,48	1,10

The parameter L* indicates the lightness or the darkness of the color. The higher the value, the lighter the color, and vice-versa. Controls from C1 and C2 are all slightly lighter than the relative hyperoxygenated wines until the "post juice racking" stage. It makes sense considering that, due to the phenolic oxidation, the musts assume color shades towards brown until clarification and racking. On the other side, after vinification and storage, the treated wines of the second batch seems to show a lighter color. Same results were found by *Cheynier et al. (1989)* on their experiment with Chardonnay musts and hyperoxygenation. The author describes the hyperoxygenated wines as browner after the pre-fermentative treatment and after fermentation. While a general lighter color of the treated wines was evaluated during the time spent in storage. This suggests that the treatment might affect the color in a positive way. Generally, signs of oxidation are given by a darker shade of the wine's color. According to these results, it seems that, after hyperoxygenation, the wines were more stable against this darkening effect. It has to be considered though, that the standard deviation values are relatively high for both groups of wines meaning that results are not very consistent and might strictly depend on the singular cases. In addition to that, looking at the first batch results, the controls appear lighter even at the "tasting stage". Furthermore, statistically speaking, significant differences were found only between stages in both batches, but not between treatments.

The parameter a* represents the contribution to the green or the red color. The higher the negative value, the higher the contribution to the green color. The contribution to the green color seems for both batches generally higher in all hyperoxygenated wines and lower in all controls.

The parameter b* indicates the contribution to the yellow or the blue color on the spectrum. The higher the value, the higher the contribution towards the yellow. In the first batch (C1) hyperoxygenated wines show higher contribution to the yellow color, compared to their controls. This confirms the values of the absorbances at 420 nm, higher in the treated wines, probably because not enough phenolics precipitated during the treatment. The hyperoxygenated wines of the second batch (C2) instead, seems to have more intense yellow pigmentation until the "post juice racking" stage, which is normal considering the darkening of the color due to the treatment. However, the contribution to the yellow color became lower after vinification and storage confirming the decrease of absorbance at 420 mn at the tasting stage. This is what it was most likely the result to expect, a less susceptibility to browning and a less intense yellow pigmentation, typical of oxidized wine.

Cab* measures the level of color saturation or brightness. The higher the value, the brighter the color. Overall it seems that the color of the treated wines it's brighter than the controls all along the stages, probably due to the precipitation of a big part of phenolic compounds. The only exception is for the "tasting" stage of the second batch (C2) where the control is slightly higher in brightness instead. The difference, in this case, is very small though and not significant.

Hab* values indicate that all wines are in the yellow-green chromaticity space, but more on the yellow side. The lower negative values of the controls indicate their position closer to the yellow ax than the respective hyperoxygenated wines. Being the values so slightly different from each other and with such high standard deviations, the differences between treatments are not significant.

What can be deducted from the CieLab analysis is that there are not very relevant differences regarding the change of color and probably they cannot be recognized simply by eye. The hyperoxygenated wines seem to have a higher contribution to the green and the yellow color plus a higher brightness. The darkness of the color is higher in the first stages of vinification but eventually, it gets lower than the controls (in the second batch). However, some of the repeats are not consistent with each other and it can be seen from the generally high values of the standard deviations. It seems that hyperoxygenation does not have a negative effect on color parameters. On the opposite, they either stayed more or less invariant or even improved in certain cases, increasing the brightness or the lightness or the contribution to the green color.

4.3.5 HRMS results

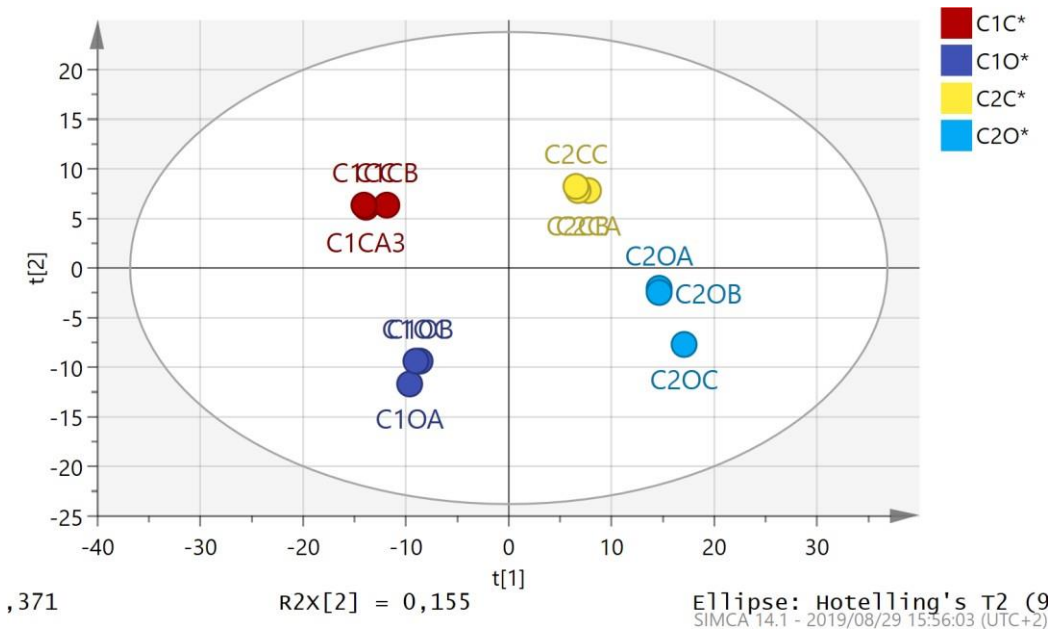


Figure 4-14. Principal Component Analysis (PCA) performed on the results related to the HRMS analysis of all repeats (A, B, C) of treated and untreated wines (O, C) from the Chardonnay batch number 1 (C1) and number 2 (C2).

Untargeted High-Resolution Mass Spectrophotometry was used to fingerprint mainly non-volatiles compounds and especially phenolics. Unlike the data obtained with the CATA analysis, where all

samples were mixed, here it is possible to distinguish separately each group of control and hyperoxygenated wines for both batches. Not only the two groups are clearly separated in the PCA, but also the two batches. Therefore, there is indeed a significant difference regarding the chemical composition of the groups. The difference is most likely due to the different concentration of phenolic compounds.

All repeats are grouped very close to each other, showing high repeatability and similarity. The only case where the repeats are slightly distant from each other is in the treated wines of the second batch (C2O) were probably the must was less homogeneously oxidized.

Even if through the sensory evaluation there was no perceived difference for astringency and bitterness between controls and treated wines, the chemical analysis shows that there was a difference regarding the compounds responsible for these parameters. The tasters might not perceive an increase or a decrease in bitterness and astringency but the oxidation and the removal of part of the phenolic compounds at the must stage did produce a change in the chemical profile of the samples.

4.4 Statistical results

For both Chardonnay batches statistical differences were found between the group of treated juices/wines and the group of the untreated ones. Some of the parameters analyzed were statistically different between stages and/or between treatments and/or considering the treatment*stage interaction.

4.4.1 Statistical analysis of treated and untreated juices and wines along winemaking stages

The statistical analysis of the parameters measured along the winemaking stages showed few significant differences, especially in regards to the interaction between treatment and stage.

The **first Chardonnay batch** shows significant differences concerning the Brix° between stages and the TA (g/L) between stages, treatments and for the stage*treatment interaction. It is definitely not surprising data considering the evolution of sugars and acidity during the winemaking process, therefore these differences are not relevant to this study.

Glutathione in both its forms was also found to be statistically different between stages, treatments and for the stage*treatment interaction. It supports the chemical results for the reduced and oxidized glutathione concentrations (mg/L) in both groups of juices/wines. Hyperoxygenated juices, in fact,

appeared to contain less reduced glutathione and more oxidized glutathione in the first stages of winemaking. The GSH, due to its antioxidant activity, undergo a faster depletion in the absence of SO_2.

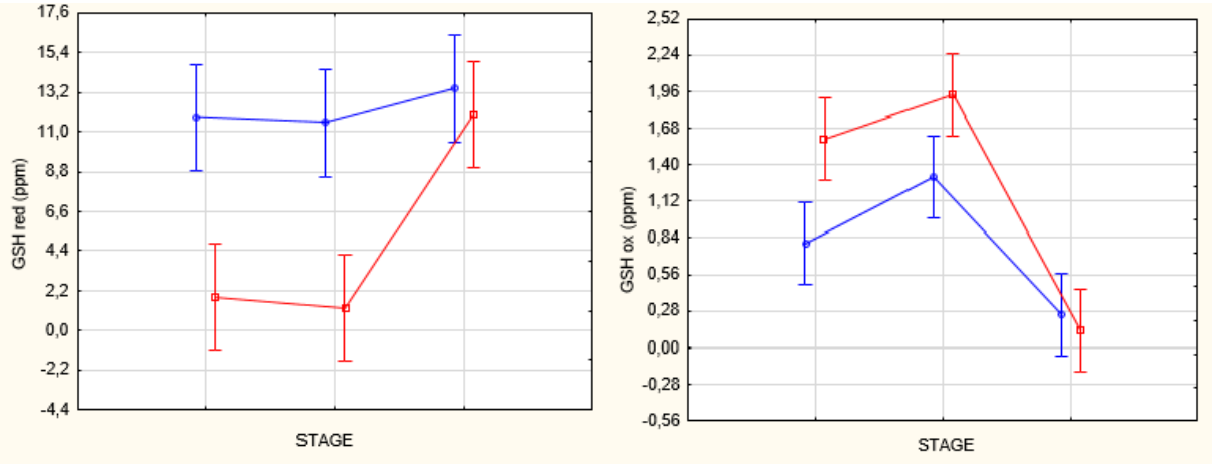


Figure 4-15. Statistical analysis on GSH red (ppm) on the left and GSH ox (ppm) on the right, for Chardonnay batch 1. Blue represents the Control juices and wines while red represents the Hyperoxygenated ones. Data taken into account were measured at the following stages: "post pressing", "post juice racking" and "bottling". For the hyperoxygenated juices/wines, the "post hyperoxygenation" stage was also included. Vertical bars denote 0,95 confidence intervals.

Colorimetric results were found to be statistically different, between treatments, for the absorbances measured at 320 nm and 420 nm. This confirms what was discussed in the colorimetric results section. Phenolic acids are significantly less in the hyperoxygenated juices/wines while the yellow pigmentation is significantly higher. Therefore, the first batch was definitely affected in its phenolic acid concentration due to the hyperoxygenation technique. On the other side, hyperoxygenation led to an increase in the yellow pigmentation of the treated juices/wines.

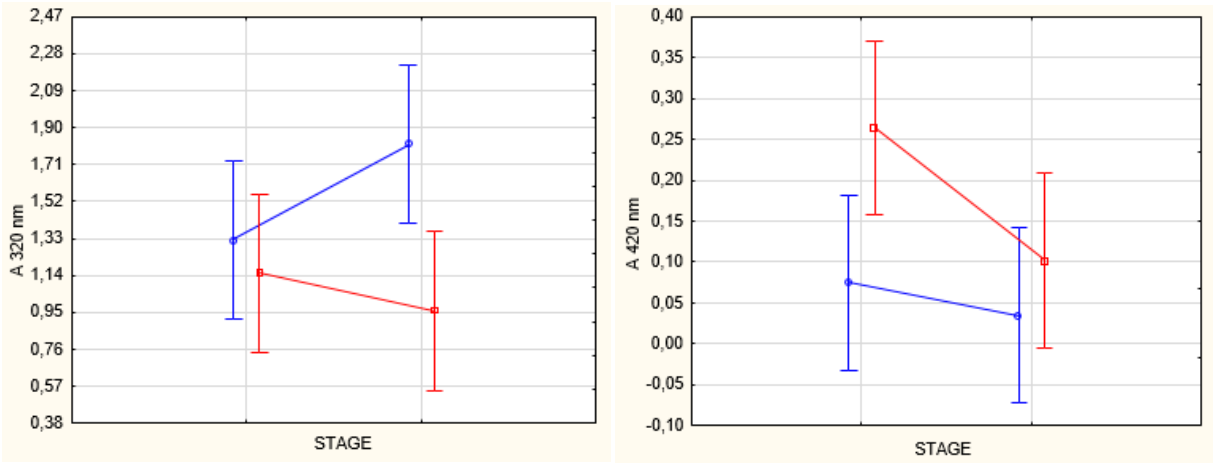


Figure 4-16. Statistical analysis on A 320 nm on the left and A420 nm on right, for Chardonnay batch 1. Blue represents the Control juices and wines while red represents the Hyperoxygenated ones. Data taken into account were measured at the following stages: "post pressing", "post juice racking" and "bottling". For the hyperoxygenated juices/wines, the "post hyperoxygenation stage was also included. Vertical bars denote 0,95 confidence intervals.

Between the CieLab parameters, L* was found to be statistically different according to the stage, meaning that the lightness or the darkness of the color changes accordingly to the different winemaking phase. It makes sense considering how clearer the wines are after sedimentation, especially in the case of hyperoxygenation when the color of the must become pretty dark and brown before clarification and racking. a*, b* and Cab* on the other side, were found to be significantly different according to the treatment. Confronting this results with the CieLab measurements, it seems that for the treated juices/wines group there is an increase of color brightness (Cab*), a significantly higher contribution to the green color (a*) and a higher yellow contribution as well (b*). The latter one supports the increased yellow pigmentation found through the measurement of the absorbance at 420 nm. These differences in color are not so perceptible for the human eye but they might become more evident with time and wine color evolution.

The second Chardonnay batch shows a significant difference for TA (g/L) between stages which again is related to the normal wine evolution, therefore, has no relevance for this study. Unlike the first batch, reduced glutathione (ppm) shows significant differences only according to the stages, while oxidized glutathione (ppm) according to the stages, the treatment, and the stage*treatment interaction.

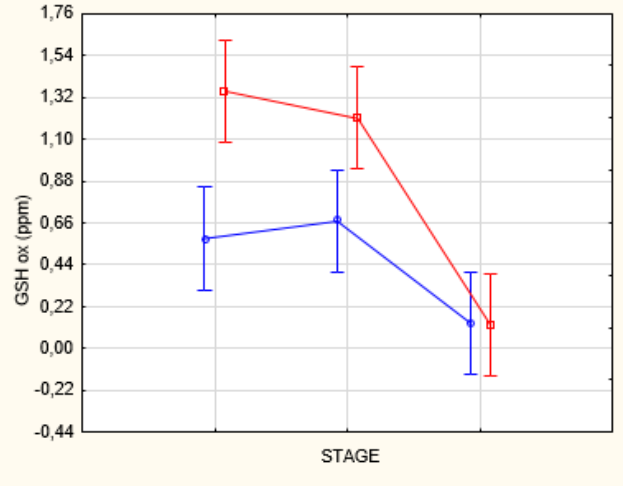


Figure 4-17. Statistical analysis on GSH ox (ppm) for Chardonnay batch 2. Blue represents the Control juices and wines while red represents the Hyperoxygenated ones. Data taken into account were measured at the following stages: "post pressing", "post juice racking" and "bottling". For the hyperoxygenated juices/wines, the "post hyperoxygenation" stage was also included. Vertical bars denote 0,95 confidence intervals.

Both absorbances at 280 nm and 320 nm are significantly different according to the stage and the treatment, while the absorbances at 360 nm and 420 nm according to the stage only. Therefore, total phenolics and phenolic acids seem to have significantly different concentrations in the two groups of wines. According to the colorimetric results, both are lower in concentration in the hyperoxygenated juices/wines, demonstrating the efficiency of phenolic precipitation following juice hyperoxygenation. On the other side, flavonoids content and the yellow pigmentation seems to be significantly different only according to the stage. It seems, therefore, that for both batches, phenolic acids were the phenolic compounds affected the most by the treatment.

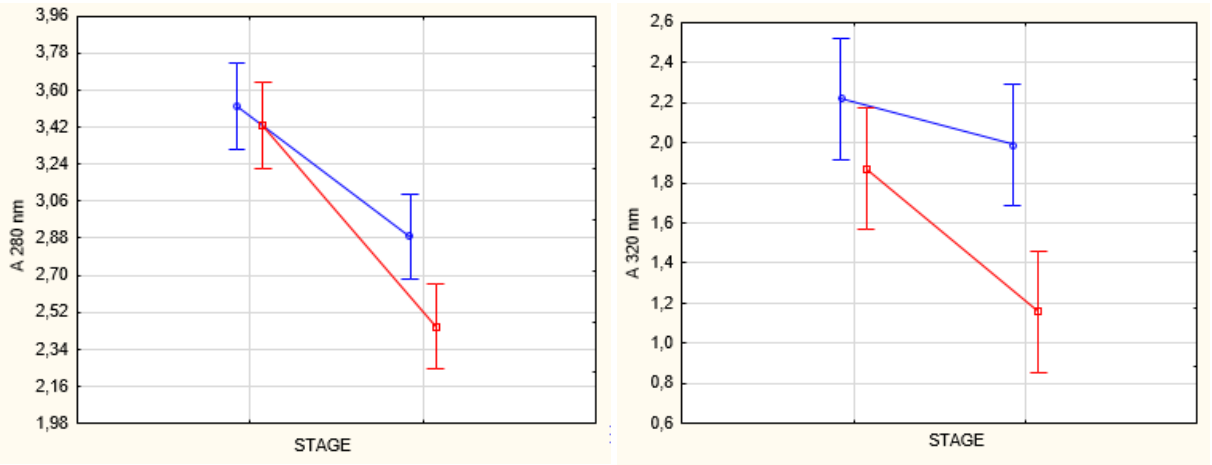


Figure 4-18. Statistical analysis on A 280 nm on the left and A 320 nm on the right, for Chardonnay batch 2. Blue represents the Control juices and wines while red represents the Hyperoxygenated ones. Data taken into account were measured at the following stages: "post pressing", "post juice racking" and "bottling". For the hyperoxygenated juices/wines, the "post hyperoxygenation" stage was also included. Vertical bars denote 0,95 confidence intervals.

Contrarily to the first batch, all CieLab parameters are statistically different only according to the stage. Being these results not similar or consistent between the two batches, it makes hard to individuate the effect related to the treatment. The results might depend on the must composition itself or it could be that the color differences are not very intense or very relevant.

4.4.2 Statistical analysis of treated and untreated wines at "bottling" stage

The statistical analysis of the results for all parameters measured via HPLC at the bottling stage also showed to be different between batches. There were very few statistically significant differences between wines. This is a positive result in term of maintaining the wine's profile and typicality, despite the different treatments.

Chardonnay batch 1 didn't present any significant differences between treatments in regards with the following parameters: Brix°, Tartaric acid (g/L), Malic acid (g/L), Citric acid (g/L), Succinic acid (g/L), Lactic acid (g/L), Glucose (g/L), Fructose (g/L), Glycerol (g/L), Ethanol (g/L) and Ethanol (%). Acetic acid (g/L) was the only parameter presenting statistical differences between treatments. The difference didn't emerge between the means of both groups of wines but it did consider all the repeats from the two groups. Checking on the chemical results, in fact, it appears that one repeat of the control wines had 0,00 g/L of acetic acid, which is probably a measurement error. All other repeats' values are around 0,2 g/L for both groups of wines, meaning that there was no relevant difference regarding this parameter for batch 1.

Concerning **Chardonnay batch number 2** instead, significant differences were found for Malic acid (g/L), Acetic acid (g/L), Glucose (g/L) and Fructose (g/L). Again, not between means but between all repeats. Acetic acid could be the case where statistical significance and practical relevance meet together, considering that the results are in line with what was found in the sensory analysis. From the CATA evaluation, in fact, VA was perceived in all control wines. On the other side, when examining the chemical results, it seems that the hyeroxygenated wines are the ones with higher concentrations of acetic acid. From the HPLC analysis, it appears that the treated wines show a maximum of 0,63 g/L and an average of 0,46 g/L while the controls present a top value of 0,18 g /L with an average of 0,17 g/L of acetic acid. All results together seem contradictive considering that the VA should have been perceived in the treated wines instead of the controls.

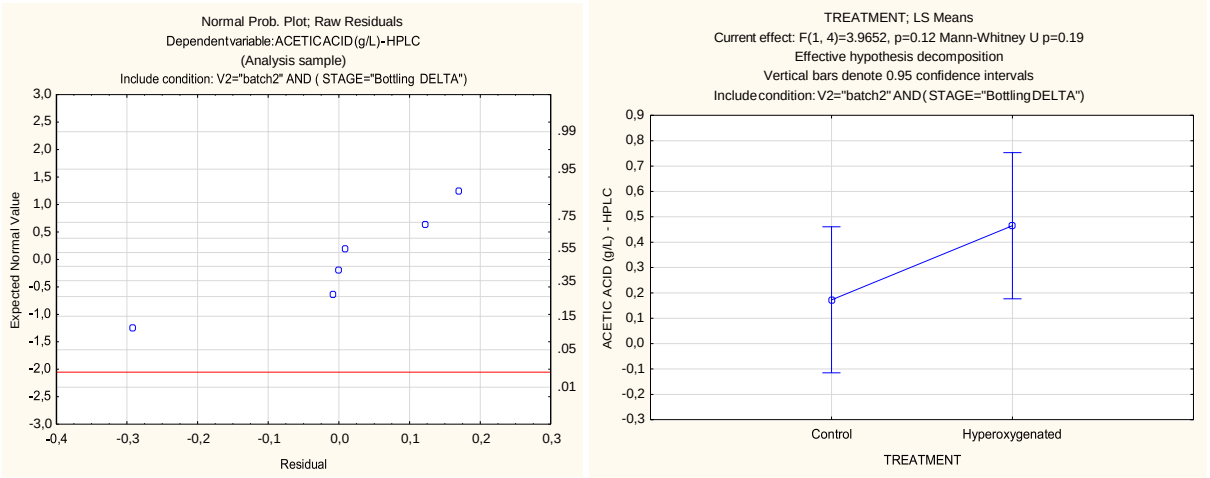


Figure 4-19. Chardonnay batch 2. Statistical analysis on Acetic acid concentration (g/L) between each single repeats on the left and between means on the right of treated and untreated wines. Data of Acetic acid concentrations (g/L) were measured at the bottling stage via HPLC. Vertical bars denote 0,95 confidence intervals.

The other statistically significant parameters between treatments, Malic acid (g/L), Glucose (g/L) and Fructose (g/L), were not perceived to be different on the sensory point of view and there were no big differences in the chemical results either. Overall the second batch was perceived to be less acid than the first one but no differences were perceived by the tasters among treatments within the same batch. Chemically speaking instead, the hyperoxygenated wines were found to be lower in both concentrations of Glucose (g/L) and Fructose (g/L) but higher in Sucrose (g/L). The difference in Glucose and Fructose, under 0,5 g/L, doesn't make a difference on a tasting and practical point of view though, and it just depends on the single fermentation's evolution of each repeat.

Statistically, the two Chardonnay batches show similar outcomes regarding the significant difference for oxidized glutathione (ppm), phenolic acids concentration (A 320 nm), and Acetic acid (g/L), even if the last one cannot be taken into account for batch 1. Confronting the statistical data with the chemical ones, it appears that the oxidized glutathione presents higher concentrations in the hyperoxygenated wines and that phenolic acids are lower in the treated wines. It is necessary though to distinguish what is statistically significant and what is practically relevant. Sometimes, even if the statistics show differences between wines, like in the case of Malic acid (g/L), Glucose (g/L) and Fructose (g/L) for batch number 2, that can be not practically relevant for the wine's aroma profile and how is it perceived by tasters. It could also be not relevant in terms of the evolution of a specific parameter on a chemical point of view. Viceversa, the decrease of flavonoids in C2O compared to C2C, can be not statistically significant but practically relevant considering that flavonoids influence the most sensory results. They are in fact primarily responsible for the development of bitterness, astringency, browning and aroma alterations during oxidative ageing in white table wines *(Schneider, 1998).*

5 CONCLUSIONS:

In light of the results from chemical and sensory analysis, we can say that the hyperoxygenation technique did produce significant statistical changes in both musts and wines. Oxygen was added throught manual aeration until the must's color reached an intense brown tone. Differences were revealed in regards to different aspects.

The parameters analysed chemically showed the effects of hyperoxygenation mainly on glutathione concentration (ppm) and phenolic evolution. At the must stage, glutathione in its reduced form was found to be statistically higher in concentration for the control juices and lower for the hyperoxygenated ones, while oxidized glutathione followed an opposite behaviour. The absence of sulphur dioxide and the oxygen supplied caused an increase in glutathione binding with the *o*-quinones to form the Grape Reaction Product. Glutathione performed its antioxidant activity until its depletion, which was faster in the treated juices.

Concerning the phenolic aspect, differences were found between batches and between treatments, according to the HRMS and Uv-Vis results. The Uv-Vis analysis enlightened the decrease in total phenolics and especially phenolic acids in all treated musts and wines from both batches. Therefore, control wines with a final higher polyphenol content, will have a higher browning potential. The results showed that overall the higher is the initial phenolic concentration, the higher is the effect of the hyperoxygenation treatment. Furthermore, a more mature batch (C2), with a lower initial content of GSH, higher polyphenol content, and higher pH, had a higher loss of polyphenols, even in the control wines. The loss of flavonoids was found to be statistically significant only between winemaking stages, regardless of the treatment. Despite that, the occurred decrease in flavonoids, especially in batch 2, could still be practically relevant in case of press juice which contains much higher amounts of this phenolic compound. Phenolic acids, as mentioned, seemed to be the phenolic compounds mostly affected by the treatment and their decrease is the most significant one also from a statistical point of view. Sensorially speaking, phenolic acids do not affect remarkably the organoleptic properties of a wine. This is probably the reason why no big differences were found in terms of bitterness, astringency and colour, the latter one evaluated by CieLab parameters. Nevertheless, the decreased absorbance values found in the hyperoxygenated juices/wines, denote the efficiency of phenolic precipitation following juice hyperoxygenation. The occurred phenolic precipitation is supported by the L* results from the CieLab calculations and the following statistical analysis. Data shows significant differences in colour darkening according to the winemaking stage. Despite demonstrating how dark the colour is compare to the control in the first stages, the lightness comes back after clarification and AF.

The yellow pigmentation was found to be significantly higher from the controls in the hyperoxygenated juices/wines of the first batch. The result is confirmed by a significantly higher yellow contribution to the colour (b*) for the same wines. Nevertheless, the outcome turned out to be positive considering that the increase in yellow pigmentation is proportionally lower over time in the treated wines, showing better stability towards colour browning. In the second batch instead, the compounds responsible for browning were initially higher and after phenolic precipitation, the yellow pigmentation decreased for both groups of juices/wines. The decrease was proportionally more evident in the treated wines meaning that the compounds responsible for that behaviour were removed and that the browning susceptibility was indeed reduced in comparison with the control wines. In conclusion, the two batches showed different behaviour in terms of phenolic evolution and an opposite behaviour in regards to yellow pigmentation evolution. The reasons behind are related to the must composition and the relative phenolic changes and evolution during vinification and wine storage. However, the behaviour in regards to hyperoxygenation was the same for both batches, showing in both cases success in the removal of phenolic compounds and a reduction in browning susceptibility.

CieLab parameters, besides L*, did not present similar or consistent behaviours when comparing results of control groups and treated groups from the two batches. The only statistically significant differences treatment-related were found in the first batch. This made it hard to individuate a pattern and the effects related to the treatment on the colour point of view. The outcome might depend on the must composition itself or it could be due to slight or irrelevant colour differences. Further studies should be carried out to evaluate the effects of hyperoxygenation on colour stability, especially after a longer period of storage. A sensory analysis should also be included to evaluate if eventual visual differences in colour might be picked up or not by the panel.

From a sensory point of view, Chardonnay's typicality was not affected by the treatment. Experts evaluated the wines slightly differently in terms of aroma profile but all samples were still recognizable as a good example of a Chardonnay white wine. Correspondence Analysis did not show evident and marked effect on the aroma profile caused by the treatment either. Nevertheless, the frequency of citation for the various aroma descriptors selected by the experts, did show few differences when describing wines belonging to the two batches and the two treatments. The second batch, with a higher ripeness state, is characterized by more frequent ‘oxidation-derived’ type of aromas in both groups of wines (treated and untreated). In addition to that, the ‘lactic/buttery’ note and ‘marmalade’ aroma were perceived more frequently compared to the first batch. The first batch, on the other hand, remained overall more citrus, fruity and flowery (‘orange blossom’ notes in particular). Differences between batches are due to the different must compositions and are in accordance with the different ripeness state of the grapes.

Treated wines were, in general, more frequently characterized by ‘citrus’ aromas, notes of ‘toasted bread’, ‘dried figs’, ‘caramel’ and ‘minerality’. In addition to that, the ‘yellow fruits’ and ‘tropical fruits’ notes were found to be in common with the control wines. Control wines were more frequently associated with flowery aromas (‘orange blossom’), yellow and tropical fruits (‘banana’ and ‘pineapple’). Furthermore, ‘apple-related’ aromas were found more frequently in the control groups, especially the ‘oxidized apple’ notes in the second batch. This demonstrates the higher sensitivity to the oxygen of the untreated wines in terms of aromatic profile, meaning that after hyperoxygenation a lower risk of getting oxidation-related aromas could be achieved. Taste and mouthfeel parameters were not perceived to be different between treatments. Between batches, only the acidity level was overall slightly lower in the second batch because of the higher maturity level.

A higher ripeness was found to be a possible responsible for an increase of the oxidation-related aromas, but to a lower extent when it comes to hyperoxygenated wines. Therefore, this technique might be a good solution in case of over-ripe grapes, to reduce the risk of developing unwanted oxidation-related aromas.

To conclude, hyperoxygenation might not be the ideal option for a producer who is prioritizing the maintenance of a certain wine style to the introduction of a different production step and its benefits. On the contrary, this could definitely be a valid option for producing a wine with a reduced sulphur dioxide content. It is also a valid alternative for press juice, which tends to have a high concentration of phenolic compounds. Phenolics could then be reduced through hyperoxygenation, making the wine lighter and easier to drink, while reducing the risk of further oxidation and the darkening of the colour during storage. Letting the polyphenols oxidize and avoid the use of nitrogen or another inert gas like CO_2 during the first stages of vinification, is a money-saving practice especially in case of large productions. At the must stage, the oxidation risk is higher due to the PPO activity, therefore, a consistent amount of inert gas is used to avoid both browning and loss of aromas. In the case of a classic method of sparkling wine production, hyperoxygenation could be a valid technique for the preparation of the base wines to avoid possible future colour instabilities and especially to reduce the SO_2 content. In fact, for this type of product, the main aromas come from the yeast activity during the second fermentation. Therefore, the primary aromas, that could potentially be affected by the treatment, play a less important role.

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7 APPENDIX

7.1 Tasting sheet used for the "Typicality assessment"

Typicality Assessment

Unwooded Chardonnay wines

Your first task is to assess a series of 12 Chardonnay wines. The wines are all made according to a standard winemaking protocol and received no wood contact. They are made from two different blocks of vineyards.

Assume you have to explain to a person, not expert in wine, what defines a Chardonnay wine. In light of that, you have to suggest to him/her to taste a wine.

For each wine presented, you will have to answer the following question:

"Do you think this wine is a bad example or a good example of a Chardonnay wine?"

Due to a variety of reasons, it is possible that a wine does not seem to you to be a good example of a Chardonnay wine. We are interested in your personal opinion.

Based on the information given to you above:

Please rate each sample on the corresponding line scale according to how close it is to be a 'very bad example' (at the left end) or a 'very good example' (at the right end) of a Chardonnay wine.

EXAMPLE:

Sample no.___584___

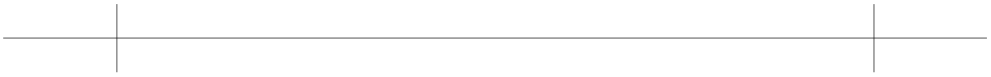
Very bad example Very good example

Now please rate the 12 samples in front of you.

Sample no. _____

Very bad example Very good example

Sample no. _____



Very bad example Very good example

7.2 Tasting sheet used for the "CATA" evaluation

Judge no._____ **Wine Code** [redacted] **Date** _____

Odours description: Choose the most relevant descriptors on the list below by ticking the corresponding box

	FRUITY				**FLORAL**		**TOASTED**
	WHITE FRUITS		**DRIED FRUITS**		Camomile		Caramel / burnt sugar
	Quince		Dried Peach		Orange blossom		Toasted bread
	Yellow Apple		Dried Fig		Honeysuckle		
	Pear		Dried Apricot		Linden Tree Flower		**WOODY**
	Oxidized Apple		Dried Apple				Oak
	YELLOW FRUITS		**TROPICAL FRUITS**		**SWEET ASSOCIATED**		**OTHER**
	Peach		Pineapple		Marmelade		
	Apricots		Banana		Glazed / Crystallized Fruit		Butter / Lactic
			Gooseberry		Cider		Mineral / Flinty
	CITRUS		Passion Fruit		Honey		
	Lemon						
	Orange						

Taste description: Choose the intensity of each taste descriptor on the list below by ticking the corresponding box

(Please make sure you mark all taste attributes)

TASTE

SWEET	Absent	Very Low	Low	Medium	High	Very High
SOUR	Absent	Very Low	Low	Medium	High	Very High
BODY	Watery	Very light	Light	Medium	Full	Very High
ASTRINGENT	Absent	Very Low	Low	Medium	High	Very High
BITTER	Absent	Very Low	Low	Medium	High	Very High
LENGTH / AFTER TASTE	Absent	Very Low	Low	Medium	High	Very High

Thank you for your time and participation