Professional Internship Report

Description and Analysis of Wine Processing Practices

Pierre Sinsollier

Dissertation to obtain the degree of

European Master of Science in Viticulture and Enology

Advisor:

PhD Manuel José de Carvalho Pimenta Malfeito Ferreira, Assistant Professor with habilitation at Instituto Superior de Agronomia, Universidade de Lisboa.

Jury:

President:

PhD Carlos Manuel Antunes Lopes, Associated Professor with habilitation at Instituto Superior de Agronomia, Universidade de Lisboa.

Members:

PhD Bruno Blondin, Professor at Montpellier SupAgro;

PhD Jorge Manuel Rodrigues Ricardo da Silva, Full Professor at Instituto Superior de Agronomia, Universidade de Lisboa;

PhD Manuel José de Carvalho Pimenta Malfeito Ferreira, Assistant Professor with habilitation at Instituto Superior de Agronomia, Universidade de Lisboa, Supervisor.

2017 - 2019

Abstract

The Vinifera European Master of Science in Viticulture and Enology program offers the possibility to conduct a professional internship coupled with a scientific dissertation as a final thesis. In the frame of this professional thesis, I was offered to work at Clos Apalta, world renown winery located in the Colchagua valley in central Chile. My role of Assistant Enologist in charge of the night shift encompassed a large spectrum of activities which, besides a managerial dimension, covered various enological manipulations.

Ensuring a permanent monitoring of the evolution of each vat and barrel is decisive, and along the course of fermentation, adjustments need to be made, starting by the preparation and inoculation of commercial yeasts. Nutrition additions are to be synchronized with cycles of cap management practices and in case of barrel fermentation, with cycles of micro-oxygenation. Regular tasting of the fermenting wines is also necessary, as it allows to detect eventual organoleptic deviation, such as the appearance of reductive aroma, volatile acidity, or volatile phenols in ageing wines. Along with the analysis of various wine-making processes employed at the winery, a few enological issues that were encountered, and their subsequent preventive or curative treatment, are also described in this report.

Clos Apalta produces solely premium biodynamic wines, with a focus on extraction, from the cultivars Carménère, Cabernet Sauvignon, Merlot and Petit Verdot. Working there represented an exceptional opportunity to learn about the caution, precision and intensity involved in the production of premium red wines aiming for long ageing potential, with the extensive use of French oak, vats and barrels, throughout wine's production. This professional thesis is a report of the observations and analysis I have performed while working at Clos Apalta, along with scientific literature reviews in order to compare the winery practices to current scientific knowledge.

Keywords: Premium wine, Barrel-fermentation, Skin maceration, Stuck fermentation

Resumo

O Vinifera European Master of Science in Viticulture and Enology oferece a possibilidade de realizar um estágio profissional juntamente com uma dissertação científica como tese final. No âmbito desta tese profissional, fui oferecido para trabalhar na Clos Apalta, vinícola de renome mundial localizada no vale de Colchagua, no centro do Chile. Meu papel de Enólogo Assistente encarregado do turno da noite englobava um amplo espectro de atividades que, além de uma dimensão gerencial, abrangiam várias práticas enológicas.

Assegurar um monitoramento permanente da evolução de cada tonel e barril é decisivo, e ao longo do curso da fermentação, ajustes devem ser feitos, começando pela preparação e inoculação de leveduras comerciais. As adições de nutrição devem ser sincronizadas com ciclos de práticas de pisas e remontagens, e no caso de fermentação de barril, com ciclos de micro-oxigenação. A degustação regular dos vinhos fermentados também é necessária, pois permite detectar eventuais desvios organolépticos, como o aparecimento de aroma redutor, acidez volátil ou fenóis voláteis em vinhos envelhecidos. Juntamente com a análise de vários processos de vinificação empregados na adega, alguns problemas enológicos encontrados e seu tratamento preventivo ou curativo subsequente também são descritos neste relatório.

A Clos Apalta produz apenas vinhos biodinâmicos premium, com foco na extração, das cultivares Carménère, Cabernet Sauvignon, Merlot e Petit Verdot. Trabalhar lá representou uma oportunidade excepcional para aprender sobre a cautela, precisão e intensidade envolvidos na produção de vinhos tintos premium, visando o longo potencial de envelhecimento, com o uso extensivo de carvalho francês, cubas e barris, ao longo da produção do vinho. Esta tese profissional é um relatório das observações e análises que realizei enquanto trabalhava na Clos Apalta, juntamente com revisões da literatura científica, a fim de comparar as práticas da adega ao conhecimento científico atual.

Palavras-chaves: Vinhos premium, Fermentação em barril, Maceração da pele, Fermentações amuadas

Resumo Estendido

O programa europeu de mestrado em Viticultura e Enologia da Vinifera oferece a possibilidade de realizar um estágio profissional juntamente com uma dissertação científica como tese final. No âmbito desta tese profissional, fui oferecido para trabalhar na Clos Apalta, adega de renome mundial localizada no vale de Colchagua, no centro do Chile. Por conta da sua localização entre a Cordilheira dos Andes e o Oceano Pacífico Sul, todo o vale de Colchagua se beneficia de uma massa de ar frio que desce das montanhas para proporcionar noites frescas, altamente benéficas para preservar a acidez das bagas. Juntamente com um alto grau de irradiação solar devido a uma latitude sul de 35°, as condições climáticas permitem um longo ciclo de maturação. Como resultado desse longo ciclo, meu estágio dentro da empresa começou por um curto período de trabalho com a produção de vinho branco na outra adega do grupo, a adega Lapostolle, enquanto as uvas dos vinhedos da Clos Apalta estava amadurecendo lentamente.

As minhas funções na adega Lapostolle englobavam a recepção da uva, prensagem, clarificação por flutuação ou sedimentação a frio, e reidratação e preparação de uma vasta gama de leveduras enológicas para inocular os mostos. Cada etapa da produção de vinho branco com a qual eu me envolvi é descrita e detalhada por uma revisão bibliográfica científica que visa justificar e analisar as práticas adotadas pela adega. Esta abordagem científica é mantida ao longo de toda esta tese.

Na adega Clos Apalta, minha função de Enólogo Assistente responsável pelo turno da noite englobava um amplo espectro de atividades que, além de uma dimensão gerencial, abrangiam várias manipulações enológicas. As uvas são colhidas exclusivamente à mão, cedo pela manhã, para que se beneficiem da temperatura fria, e transportadas para a adega em pequenas caixas, para evitar o esmagamento desnecessário das bagas, o que teria um impacto negativo na qualidade do vinho. Continuando com a recepção da uva na adega, a orientação qualitativa do processo de vinificação é assegurada por um desenrolamento suave e uma cuidadosa selecção de bagas à mão. Transportado por um carrinho de rolamento, a transferência das bagas para as cubas não requer o uso de nenhuma bomba. Lá, protegido com dióxido de enxofre e adicionado com enzimas pectinase, a maceração pode começar. Várias práticas envolvidas na maceração da pele são descritas, juntamente com

referências científicas, e sua influência na extração de compostos fenólicos e precursores aromáticos é discutida.

Assegurar um monitoramento permanente da evolução de cada tonel e barril é decisivo, e ao longo do curso da fermentação, ajustes devem ser feitos, começando pela preparação e inoculação de leveduras comerciais. As adições de nutrição devem ser sincronizadas com ciclos de práticas de pisas e remontagens, e no caso de fermentação de barril, com ciclos de micro-oxigenação. A estratégia de nutrição pode influenciar significativamente o perfil organoléptico do vinho resultante e, portanto, é necessário um cuidado atento. A falha em fornecer à população de levedura um substrato de nitrogênio suficiente pode resultar em desvios sensoriais, como aroma redutor e perda de caráter frutado. Consequentemente, a importância da nutrição de levedura, o momento de sua adição e a natureza dos recursos nutritivos enológicos disponíveis para o enólogo também são discutidos neste trabalho. Além de antecipar a exigência de levedura para realizar eficientemente a fermentação alcoólica, também é necessário degustar regularmente os vinhos depois de fermentados, pois permite detectar eventuais desvios organolépticos, como o aparecimento de aroma redutivo, acidez volátil ou fenóis voláteis em vinhos envelhecidos.

Juntamente com a análise de vários processos de vinificação empregados na adega, alguns problemas enológicos encontrados e seu tratamento preventivo ou curativo subsequente também são descritos neste relatório. Durante a vindima de 2019 em Clos Apalta, a principal questão que enfrentamos foi uma grande proporção de fermentações amuadas, cuja causa ainda está por determinar, mas há evidências de uma relação glicose / frutose nas bagas desfavoráveis à levedura *Saccharomyces cerevisiae*, em anos quentes como 2019 foi. Neste contexto problemático, técnicas como "Pied-de-cuve" foram empregadas como uma tentativa de restabelecer a fermentação alcoólica e completar o consumo de açúcar. Em circunstâncias de fermentação paralisada, a proliferação de bactérias do ácido acético e o aumento resultante da acidez volátil é um risco comum. A levedura de desenvolvimento lento *Brettanomyces bruxellensis* também pode proliferar sob tais circunstâncias, e a aparição resultante de fenóis voláteis pode deteriorar o perfil sensorial do vinho. Em relação a um amplo espectro de fatores de deterioração do vinho, a ação preventiva do dióxido de enxofre está bem documentada, mas a ação curativa da quitosana em relação a *Brettanomyces bruxellensis* também é discutida nesta tese. Exemplos de vinhos com o detalhe das manipulações aplicadas a eles, e sua descrição química e sensorial, também são expostos para ilustrar a produção da adega para esta vindima particular de 2019.

A Clos Apalta produz apenas vinhos biodinâmicos premium, com foco na extração, das cultivares Carménère, Cabernet Sauvignon, Merlot e Petit Verdot. Trabalhar lá representou uma oportunidade excepcional para aprender sobre a cautela, precisão e intensidade envolvidos na produção de vinhos tintos premium, visando o longo potencial de envelhecimento, com o uso extensivo de carvalho francês, cubas e barris, ao longo da produção do vinho. A prática da fermentação em barril no caso das uvas tintas foi uma descoberta completa, e o trabalho em barril representou um novo conjunto de habilidades que eu tive a chance de desenvolver enquanto trabalhava lá. Esta tese profissional é um relatório das observações e análises que realizei enquanto trabalhava na Clos Apalta, juntamente com revisões da literatura científica, a fim de comparar as práticas da adega ao conhecimento científico atual.

Table of Content

List of Figures

List of Tables

List of Abbreviations

4-EP: 4-ethylphenol

4-EG: 4-ethylguaiacol

AAB: Acetic Acid Bacteria

CM: Carbonic Maceration

DAP: Di-ammonium Phosphate

LAB: Lactic Acid Bacteria

MLF: Malolactic Fermentation

MOG: Material Other than Grape

NTU: Nephelometric Turbidity Units

OIV: International Organization of Vine and Wine

TA: Titratable Acidity

VA: Volatile Acidity

VSP: Vertical Shoot Positioning

YAN: Yeast Assimilable Nitrogen

1. Introduction

The aim of the Vinifera European Master of Science in Viticulture and Enology is to train the future generation of researchers and executives from the worldwide wine industry. Provided a solid background in the various fields of the industry, the future graduates must be able to analyze with relevance and adapt to particular situations encountered within wine production, vineyard's management, or even wine trade, marketing and business. The program aims at forming professionals able to manage a team and ensure the rightful and efficient operation of a company within the wine industry. The final thesis concluding the 2 years' program represents an exceptional opportunity to give one's career the orientation one desires, and it allows the future graduate to specialize in one (or more) fields of the industry, in order to improve his/her understanding and knowledge. This opportunity can be considered as a decisive turning-point of one's career, as it defines the direction aimed for, whether it is towards research, viticulture, enology, or even management, business and education.

Particularly interested in the fields of enology, I decided to take this opportunity of specialization and optimize the learning outcome of this experience by realizing a professionalizing internship within a winery. My goal, as a professional, is to develop my skills and open mind through various harvests in different countries, and aim for a consulting activity in the future. These several professional experiences in various countries help improve my understanding of the wide range of challenges faced by enologists and viticulturists around the world. Indeed, climate change seem to affect each wine producing country/region in different ways (shortening the growing cycle of vines, intensifying drought or spring rains, delaying or hastening budburst, occurrence of spring frosts, hail, heat waves) and the composition of grape berries is strongly affected by the plant's phenological response to these events. Consequently, the role of the enologist is to adapt to these modifications and optimize the quality of the wine to be produced, each vintage, in each particular "terroir". Understanding the challenges faced by enologists in various areas of diverse climates (cool, warm, dry, wet) appears to me as a necessity to perform efficiently as a consultant, in the future.

As a result, after previous harvest experiences in Australia, South Africa, Portugal, it is in Chile that I decided to work and develop this final thesis. With a unique situation, between the Andes mountains and the Pacific Ocean, Chile offers a huge diversity in terms of soil composition, mesoclimates, stretching from the cold and wet Patagonia in the south, to the large Atacama's desert in the north. The entire center of Chile is composed of successive valleys (Maule, Curicó, Colchagua, Maipo, Casablanca are amongst the most famous), offering numerous slopes onto which vineyards have been implemented. It is in the Colchagua valley that I had

the chance to work, and more specifically in the Apalta valley, secondary valley within the Colchagua region. After a short period spent at the Lapostolle winery to help with white wine making, I rapidly took on the duty which I was hired for at Clos Apalta winery, from the same company Lapostolle-Marnier. Recognized as one of the best wineries in the country, working at Clos Apalta was an extraordinary opportunity to learn about premium wine production with a focus on extraction, concentration and intensive oak ageing.

This work is a report of the observations and analyzes I have performed while working for the Lapostolle company, mostly at Clos Apalta, along with scientific literature reviews in order to compare the winery practices to current scientific knowledge.

Figure 1. Picture of Clos Apalta winery (and hotel) from the vineyards.

2. Description of the Duties

When the enologist of Clos Apalta first contacted me, the position she was offering was a Cellar Hand's position, with duties covering reception, cellar work, tending to wines. Reception encompasses, amongst other roles, taking part of grapes reception from the fields and organizing the reception area with careful tracking of the different plots, ensuring the transfer of the harvest to the reception line where the grapes would be destemmed and sorted, ensuring synchronization between the reception line and the transport of grapes to the fermentation room. Cellar work is the main activity, and encompasses temperature control, cap management such as punch-downs, pump-over and délestages, rotation of barrels, cleaning of vats, transfer of barrels and overall maintaining of the cleanliness of the fermentation room. Tending to the wines concerns mostly the wines undergoing barrel ageing, which needs regular topping up, racking from the lees, sulfur correction and cleaning of barrels. This Cellar Hand role covers the entire process of winemaking, from grapes to wine, and offers a wide spectrum of roles which enables a thorough understanding of the production.

However, after reviewing of the profile of each intern, the winery's direction decided that my qualifications and experience were relevant for a role of team management, and they offered me the position of Assistant Winemaker, responsible of the night team. This role covers a large range of topics, from management of the team in their respective duties to winemaking decisions and enological manipulations. Temperature control and density measurements ensure a permanent tracking of the evolution of each vat and barrel, and along the course of fermentation, adjustments need to be made, starting by the preparation and inoculation of commercial yeasts. Nutrition additions along with their calculations are to be synchronized with cycles of punch-downs and pump-overs, and in case of barrel fermentation, with cycles of micro-oxygenation. Regular tasting of the fermenting wines is also necessary, as it allows to detect eventual organoleptic deviation, such as the appearance of reductive aroma, volatile acidity, or volatile phenols in ageing wines.

This Assistant Winemaker role helped me understand the complex connections between each step of the winemaking process, and clarify many aspects of the enologist's responsibility. The management of a team of 6 people was a challenge I was happy to take on, and understanding the compromise between availability and authority was definitely an important learning outcome from this experience. Working along 2 experienced enologists was a precious chance to learn specific methods of winemaking, avoid mistakes, and get inspired by different philosophies and approach to enology, and I am grateful for the trust they demonstrated and the opportunity I was offered.

3. Viticulture Analysis

Clos Apalta vineyards are entirely located in the Apalta Valley, which is a secondary Valley within the Colchagua Valley, stretching from the foothill of the Andes to the Pacific Ocean. Although the coast of Southern and Central Chile is strongly affected by the Humboldt current, the valleys are somehow partially protected from it and benefit from a large diurnal thermal amplitude. Indeed, the Humboldt current is a cool air current flowing along the Southern Pacific Ocean and, blocked by the natural wall formed by the Andes mountainous chain, it flows North along the coast of Chile. Inland, and covered by mountain chains, the Apalta valley benefits from long days during the ripening months, due to a latitude of 35° South, hence a high degree of irradiation during summer. From its location between Andes and Ocean, the entire Colchagua valley benefits from a mass of cool air flowing down from the mountains to provide cool nights, highly beneficial to preserve acidity in the berries and, together with the degree of irradiation, allow a long ripening cycle. The rainfall is very low during the ripening season and this aridity also favors the potential of quality from the grape berries produced in such conditions. However, most of the vineyard of Clos Apalta (as well as Lapostolle for that matter) are equipped with a drip irrigation network, to prevent from excessive water deficit during the dry months of summer, and balance with the disparity of rain along the year, in average 550mm.

Figure 2. Picture of the East side of the valley, planted with vineyards on slopes.

Located entirely on slopes, the vineyard of Clos Apalta is managed with a focus on fruit's concentration. Beside the aridity, high thermal amplitude and cool nights previously mentioned, the grapevines are planted (mostly in vertical planting along the slope) on South and East facing slopes, to limit the intensity of irradiations during the ripening season, enabling a slow accumulation of sugar which, in turns, allow ripe fruits to have a high aromatic and phenolic content, once full maturity is reached. The cool nights help preserve acidity even at full maturity which offers a large window for the harvest, once grapes have reached the desired chemical profile. The low rainfall during late summer adds to such unique length of the window for a qualitative harvest.

The slopes are dominated by a sandy loamy granitic soil, with a low content of clay, of medium depth on the mother rock (granitic). Some areas are deep, still with a loamy granitic soil, and confer to the vineyard a higher vigor, hence the choice of planting Merlot on those plots, to promote vegetative development of this early variety and therefore delay the fruiting season to harvest later and avoid harvesting at the pick of summer. Most of the Cabernet Sauvignon is planted on a granitic colluvial soil described as "redoxic". This term describes soils presenting areas with a high content of ferric oxide ions (Fe^{3+} of red colour), testifying of oxidative condition related to dry prolonged periods, and areas with a high content of ferrous iron (Fe^{2+} of blue/green colour), sign of reductive conditions, in the presence of water. This redoxic type of soil is witness of periods with water logging alternated with arid periods. Usually, the soil water capacity is fulfilled during winter rains, and the dry summers applies extended aridity conditions. Cabernet Sauvignon performs well in these conditions as it benefits from consequent water reserves during spring and very dry conditions during fruiting and ripening seasons, shortening its usually long cycle. Carménère and Petit Verdot tend to have a very long growing cycle, often described as very late varieties (Boursiquot *et al.,* 2009). For this reason, they are planted on poor soils, of low depth on the mother rock, and as a result, their cycle is shortened and they can be harvested before the autumn rains, usually 2 to 3 weeks after Cabernet Sauvignon.

The soil's relative low fertility in all Clos Apalta vineyards, located on slopes, is complemented by a high density of plantation comprised between 5681 and 6666 plants per hectare. Along with adapted viticultural practices such as fruit thinning, the aim of the management of the vineyards is to maintain low yields, improving concentration of flavor in the berries. The average yield varies between varieties, but is maintained between 3 and 4 tons per hectare. Parts of the vineyard is dedicated to old vines, with 12.28 hectares of Cabernet Sauvignon planted in 1920, 13.14 hectares planted in 1940, and 1 hectare of Carménère planted in 1930, all from massal selection.

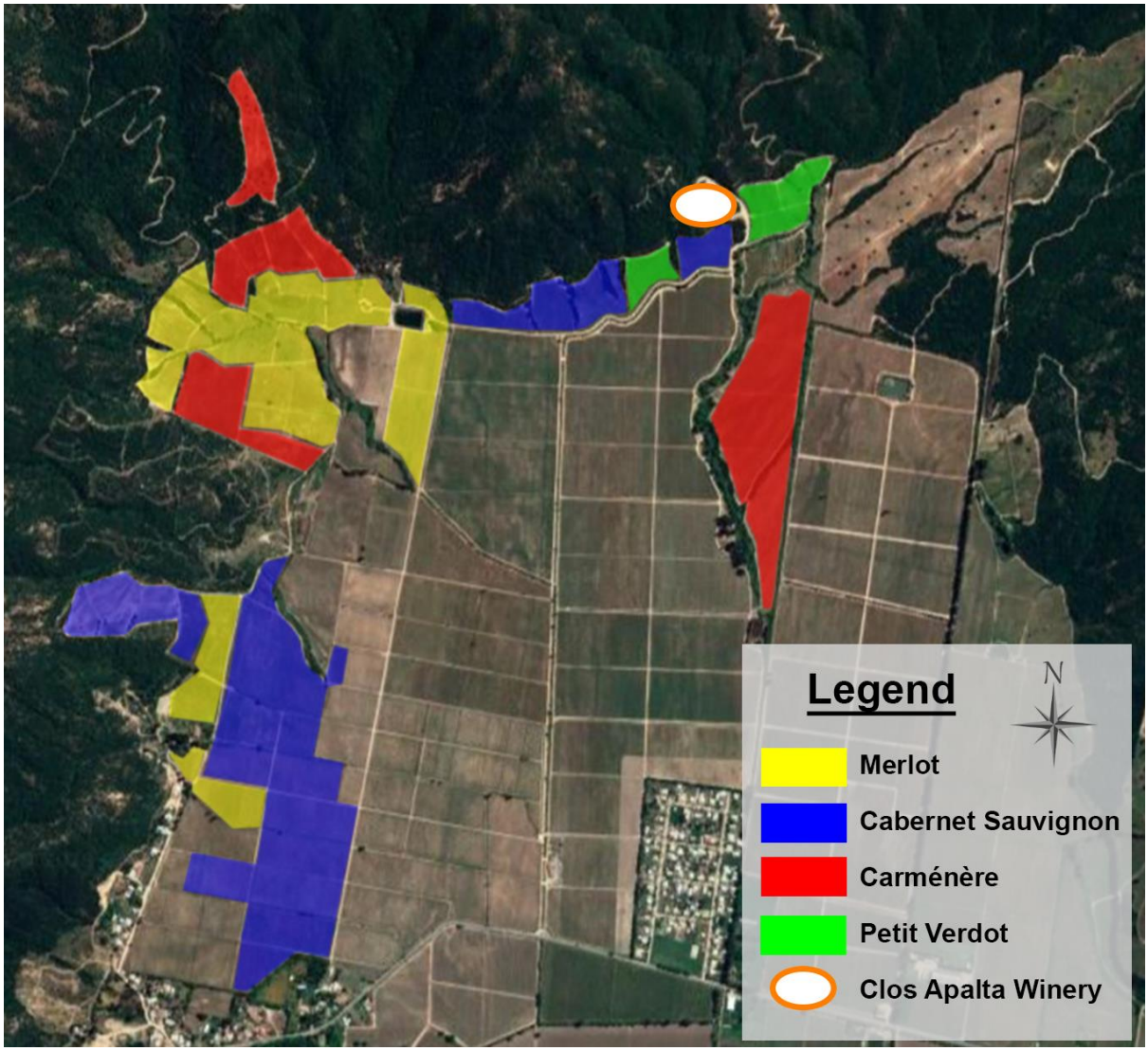

Figure 3. Aerial map of Clos Apalta vineyards, with cultivar repartition.

All varieties planted at Clos Apalta are originated from the Bordeaux area, as Lapostolle is a French company aiming at producing, here in the Colchagua Valley of Chile, some premium wines able to compete with famous Châteaux of Bordeaux, especially the Médoc area known for its heavily oaked blends. As a result, concentration of flavors instead of high yields is desired on the plots of Clos Apalta, in opposition to the central part of the property, more fertile colluvial soils with higher content of clay, which produce grapes for Lapostolle winery, focused on larger volumes. Clos Apalta vineyards are planted with the Bordeaux red varieties Cabernet Sauvignon, Merlot, Carménère and Petit Verdot. Most vineyards are planted on their own roots since Chile did not suffer the infestation of *phylloxera*, the grapevine pest that devastated European vineyard in the second part of the 19th century (Keller, 2015). Few plots are grafted

on 3309C or 101-14 Mgt rootstocks, both crossings of *Vitis Riparia* and *Vitis Rupestris,* susceptible to chlorosis but the soil is deprived of limestone. The use of these 2 rootstocks confers to the grapevine a higher tolerance to water logging (especially used for Cabernet Sauvignon planted in redoxic soil).

All vineyards are trained in Vertical Shoot Positionning (VSP) for ease of manipulations, although the entire vineyard work is done by hand. Carménère is pruned as a double Guyot cane pruning to counteract its low basal bud fertility (Gutiérrez-Gamboa *et al,* 2018), and requires basal leaf removal, in order to lower its methoxypyrazine naturally high content (Botezatu *et al,* 2016). Cabernet Sauvignon is pruned as Single Guyot as its vigor is rather high and maintaining a moderate bud load is desired. Merlot and Petit Verdot are head/spur pruned or Single Guyot is used on some plots. For all varieties, fruit thinning is systematically performed, to lower the fruit load of each plant and improve aroma's concentration by lowering the yield.

Table 1. Summary of the area under vine in Clos Apalta vineyards, in hectares.

Variety	Area (ha)
Cabernet Sauvignon	25.66
Merlot	21.71
Carménère	16.39
Petit Verdot	4.71
Total:	68.47

Clos Apalta vineyards have been managed in a biodynamics system since 2008, and is fully certified by CERES and Demeter since 2011. The winery produces its own compost, and fertility of each parcel of the vineyard is controlled by its utilization along with adapted cover cropping, mainly legumes such as clover, to take advantage of winter rains and enrich the soil in nitrogen. The use of bio-stimulant preparation, such as decoctions of Yarrow (preparation 502), Chamomile (503), Nettle (504), Oak bark (505), Dandelion (506) or Valerian (507) are also used, dissolved in water and sprayed on the soil. No pesticides or synthetic fertilizers are employed, nor any machinery in the vineyards. The winery also keep tracks of the entire Carbon emissions related to the production, from its waste and electric consumption, to storage and shipping of its wines around the world, and finances each year organisms planting forests. Through this Carbon footprint offsetting, the winery is certified Carbon Neutral.

4. Winery Description

Clos Apalta was designed with concern for both the quality of the wines they will produce, and their environmental impact. To comply with both parameters, a design based on gravity practices was agreed on, as it limits the use of pumps, and such gentle processing of grapes, musts and wines is consequently beneficial to the final product (Christmann & Freund, 2010). The environmental impact's benefit of this gravity design is, beside lowering drastically the need of electrical consumption by pumps, but also reducing the need of cooling by air conditioning devices as temperatures are low and stable underground. This feature permits to age the wines in a cool cellar, where temperature and humidity control is less intensive than in an above-ground cellar, thus leading to lowering energy consumption and production costs. Nevertheless, practices like remontage or délestage still require the use of pumps, although it is very limited throughout the production.

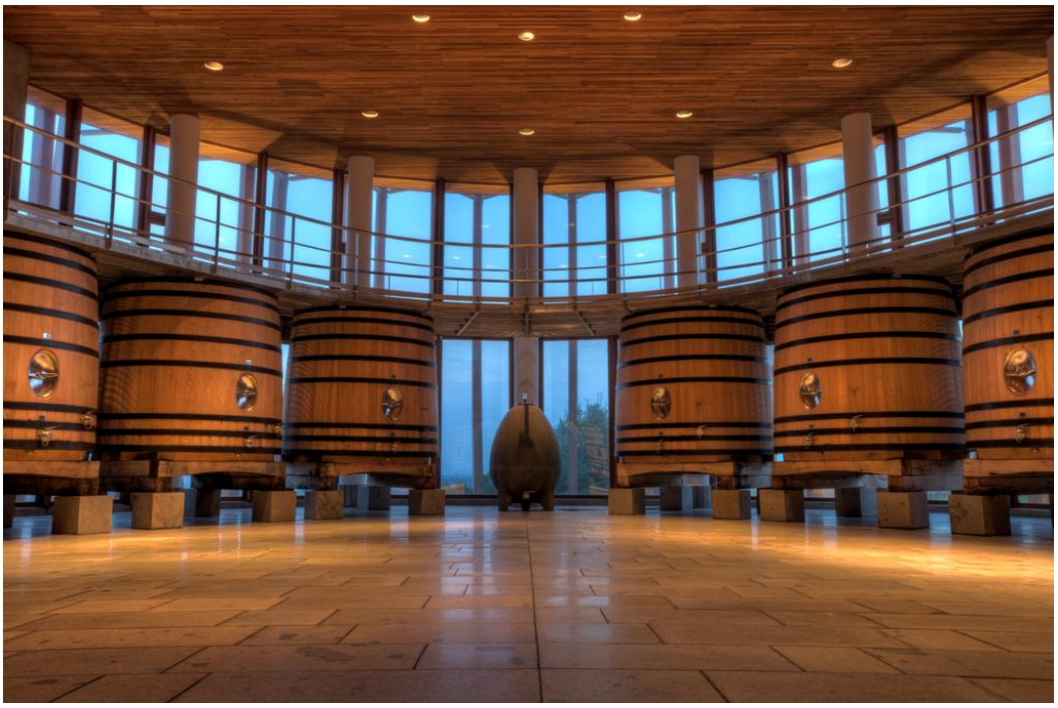

Figure 4. Picture of the fermentation room.

Transferring wine from the fermentation room to the floor below called "Primero año" (Spanish for "First year"), where the wines will remain for 12 months and where malolactic fermentation will be performed, can be done without pumping. At 4 different locations in the fermentation room, a metallic valve was embedded in the ground, and leads to the floor below. Connecting

a hose to the valve, and another hose downstairs can allow to transfer wine from vat or press upstairs to barrels downstairs by the simple action of gravity. This idea was reproduced between the “Primero año” and the floor below it, called “Segundo año” (Spanish for “Second year”) where the wines will be aged for 12 more months before being blended and bottled in the room called “Tunnel”. This lower room comports a large door allowing a truck fitted with a bottling line to enter. Indeed, the winery is not equipped with its own bottling line but uses the services of a mobile bottling line company, once per year.

The winery is embedded in a hill of 30 meters of altitude, and for this reason, it is possible to access it from the Reception floor (on the same floor as the platform on top of the wooden vat, allowing to fill the vats without pumping) or from the Tunnel, 4 floors below. A circular access road reaches both entrance points of the underground winery.

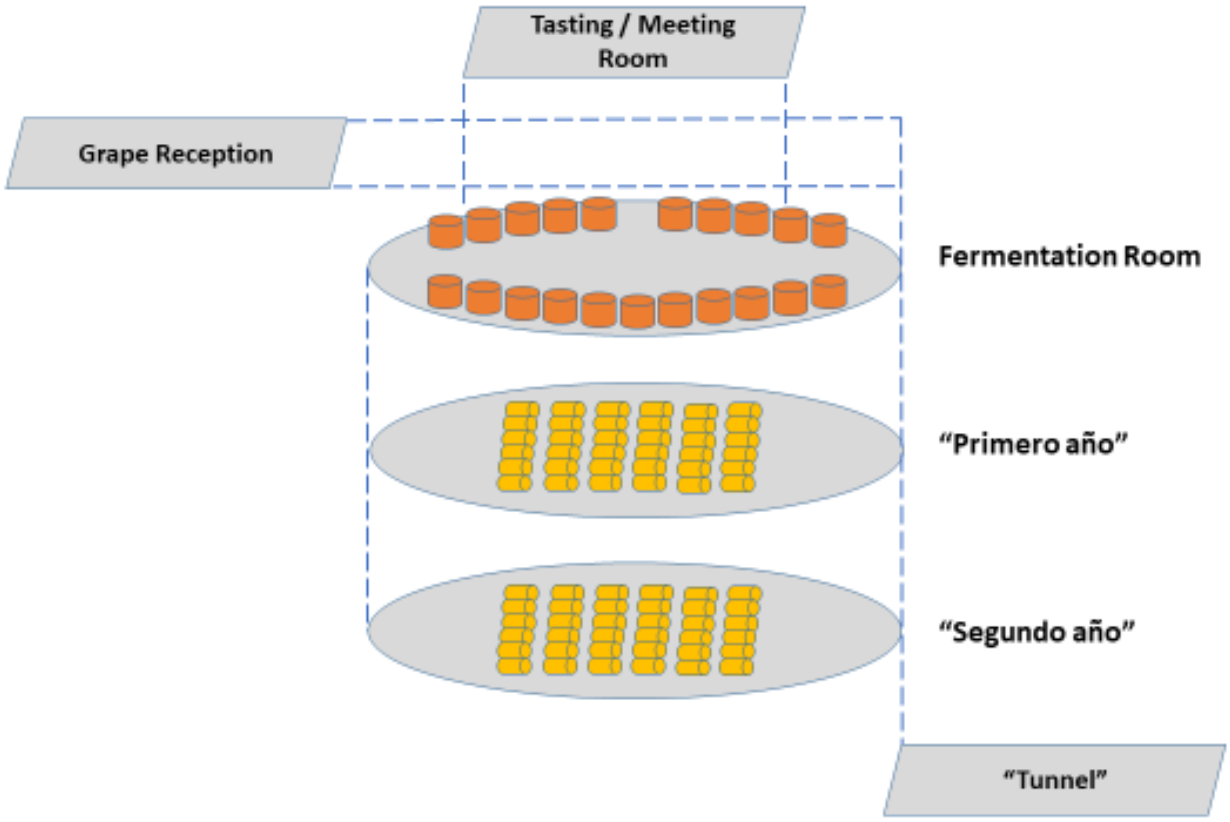

Figure 5. Schematic representation of the 6 levels of the winery.

5. Description of Clos Apalta Wine

Chile is worldwide known for the emblematic variety which gave the country its reputation in the 1970's, Carménère, but Cabernet Sauvignon is by far the most planted variety in the country. At Clos Apalta also, Cabernet Sauvignon is the dominating variety, although their premium wine blend has a Carménère dominant component. Carménère provide the rich structure of the blend, with powerful tannins and ripe black fruit flavor, balanced by the freshness and acidity of Cabernet Sauvignon. Merlot brings roundness, body and fruits to the blend, while the minor component Petit Verdot offers spice and colour. According to the climate of each particular year, the repartition is adjusted to express the "terroir" while ensuring impeccable balance. This challenge will determine most winemaking techniques employed, and their adjustments year by year. Fermented in 75hL French oak vat from the cooperage François Frères, or in French oak barrels from various producers (Saury, Seguin Moreau, Radoux, Ana Selection, Orion, Baron, Sylvain), the impact of oak on the wine's organoleptic characteristics is intense, as the wines are not meant to be consumed young, but instead aim for a long ageing potential. Clos Apalta has been very successful since its creation, as it received numerous international awards and prices. Indeed, the 2005 vintage received the title "#1 Wine in the World" by Wine Spectator, while Wine Enthusiast awarded the winery "New World Winery of the Year" in 2008, or even the wine critic James Suckling decorated it with a 100 points evaluation for the 2014 vintage. With such reputation and ageing potential, Clos Apalta wines are regarded as premium quality, with the 2014 vintage currently selling for the price of 215$ at the boutique of the winery. The production varies according to the vintage, but usually lays between 52,000 and 65,000 bottles per year. The second label of the winery, called "Le Petit Clos", represents each year between 30,000 and 45,000 bottles.

Figure 6. Picture of a bottle of Clos Apalta 2014.

6. White Wine Production at Lapostolle Winery

6. 1. Reception

Lapostolle winery processes both machine harvested and hand-picked grapes, as it aims at offering a large range of wines, from youthful easy-drinking fruity wines to premium wines with long ageing potential. To respond to the requirements of both ends of the spectrum of wines they produce, the reception is composed of 2 distinct lines, entirely independent from each other. Reception line “number 1” (or “Linea Una”) is adapted to large volumes to process machine harvested grapes. It starts by a large hopper with a conveyor screw to push the grapes to the conveyor belt situated at its exit. The belt carries the grapes to the horizontal centrifugal destemmer, where remaining pieces of stems and other MOG (Material Other than Grapes) will be removed. The destemmed grapes are then pushed by a pump through a large hose, carrying them either to the pneumatic press, in case of white wines, or to stainless steel tanks after an additional step of crushing in case of red wines.

The second reception line (or “Linea Dos”) is only designed for hand-picked clusters, as it starts by a small hopper where the reception staff empties either the 20 kg boxes used for transportation of the clusters from the vineyard to the winery, or the 300 kg bins by the aim of a forklift, in case of large containers being used for transportation. This line is equipped with a Vaslin-Bucher Delta Oscillys® model 200 (vibrating tangential destemmer) paired with a Delta Vistalys® R2 (optical sorter with pressurized air sprays) and a cooling chamber with an adjustable crushing unit at its exit. This high performance reception line allows a more selective and efficient grape sorting to ensure that only healthy, unburnt, intact berries are selected for the production of premium wines.

6. 2. Pressing

After reception, white grapes are transferred to the pneumatic press (Vaslin-Bucher® XPlus 50, capacity of up to 10 tons of destemmed grapes) and protected as soon as possible with Dry Ice, allowing a layer of CO_2 to blanket the berries and protect the must from oxidation. Until the press is filled and ready to close, protecting the grapes is a priority. It is also possible to add SO_2 in the press to protect the free run juice, especially in case of berries crushed at reception to allow some skin contact, although most SO_2 will be flowing out with the first free run juice and won’t protect the juice later released from the crushed berries. Press cycles will

depend on the grape variety (thickness of the skin, tendency to release high amounts of solids into the must) and on the wine profile targeted (sparkling or still white wine).

The sparkling cycle allows a limited amount of solids in the must, a lower colour extraction and a lower impact of oxidation on both colour and aromas. Its principle is to press the grapes with sequential rise of pressure from 0 bar to 1.6 bars, alternating a pressure build-up phase (triggered by an integrated air compressor filling up the membrane chamber) with a pressure holding phase of specific duration, before a partial rotation of the press chamber. This sequence is repeated with, each time, a rise in pressure until the maximum pressure target has been reached (Jégou *et al.,* 2017).

The standard press cycle follows the same principle, but integrates a phase of decompression (until -0.05 bar) before rotation of the press, allowing the mass of grapes to change its spatial configuration, in order to apply pressure on each berry under a different angle. This cycle allows to reach higher pressing yield, but increases the amount of oxygen in contact with the berries, and thus increases the degree of oxidation of the must over time, all through the pressing cycle (Freund *et al.,* 2008).

It is important to mention that the sparkling cycle is also well adapted to whole bunch pressing, recommended to extract colourless juice from red berries (Jégou *et al.,* 2017). This pressing method can improve must quality from varieties with fragile stems / pedicels such as Riesling, in which case destemming is not advised when a fresh, aromatic profile is desired. Particles of stem would negatively affect the aromatic profile of the wine, adding undesired green, herbaceous notes (Freund *et al.,* 2008). The sparkling cycle is also recommended for varieties with a tendency for high turbidity of musts, such as Sauvignon Blanc. Clarification of the turbid must can be difficult, even with high doses of clarification enzymes, and time consuming in case of cold sedimentation. Therefore, avoiding the previous step of destemming and opting for a gentle sparkling press cycle can improve the resulting must turbidity and facilitate its clarification. For turbid musts, or for sparkling production, it is also advised to discard the first 300L of free run juice, which contains a very high amount of solids, dusts, and other undesired particles (Blanck & Valade, 1989).

6. 3. Clarification

After pressing, the resulting turbid must needs to be clarified in order to remove the coarse sediments, solids from the skins and stems, dust, pesticides residues and eventual materials

other than grapes (MOG). These sediments would impart to the wine undesired organoleptic characteristic, such as bitterness or unripe and green aroma (Christmann & Freund, 2010). This step of clarification can be achieved by 2 mains pathways in the winery: Cold Sedimentation and Flotation. Other wineries can also choose to clarify the must by Centrifugation, or less commonly by Filtration.

Cold Sedimentation consists in allowing, over time, the heavy compounds in suspension in the must to precipitate under the influence of gravity. This can be achieved by placing the must in a container, here in stainless steel tanks equipped with cooling jackets. Temperature is maintained between 10°C and 12°C to limit risks of oxidation, microbiological spoilage, or unwanted start of fermentation by wild yeasts. The addition of SO_2 at concentration between 3 – 5 g/hL can provide with antiseptic properties that limits microbiological spoilage risk during this process (see 8.5. Sulfur Dioxide). The efficiency of the sedimentation process depends mostly on the must's viscosity, which is related to sugar content, presence of pectins or presence of β-glucans produced by botrytis cinerea for instance. Phenomenon of flocculation of particles will also significantly influence the speed of sedimentation, as coarse particles tend to settle faster than fine particles, according to Stoke's law. Electrostatic repulsion between particles whose outside layer carries similar charges limits flocculation, and maintain particles in suspension (Spasic, A. M. 2018). The addition of fining agents such as Gelatins, Egg Albumine or Casein can largely improve the flocculation effect and thus, reduce the duration of the sedimentation process (Boulton *et al.,* 1999). After a period that depends on the possible additives, and on the initial turbidity, the turbidity of the must can be measured again. The turbidity target, usually reached within 24h, is between 100 and 250 NTU (Ribéreau-Gayon *et al.,* 2006a). Below 100 NTU, the must is considered slightly deprived of lipids (sterols, unsaturated fatty acids) which will result in the production of unhealthy yeast membranes, and issues along the course of alcoholic fermentation, due to a limited ethanol tolerance (Fornairon-Bonnefond *et al.,* 2002). Over 250 NTU, the must is not considered sufficiently clarified, as many particles detrimental to wine aroma are still in suspension (Ribéreau-Gayon *et al.,* 2006a). Once the target of turbidity has been reached, the must can be racked to a different tank, and the lees can be either discarded or disposed in a tank, together with lees from other musts. Added with high content of SO_2, here 7 g/hL, and kept at the temperature of 10°C, the lees will later be processed to extract clear must in order to maximize the yield, thus increasing the profitability of the winery.

Flotation is performed by a specific device, here the E-flot 50 by AEB engineering manufacturer. Unlike the process of cold sedimentation, flotation doesn't require an extended period of time, and it is possible to perform this operation continuously after pressing the

grapes, before transferring the clear must to the tank where fermentation will take place. Also called “Reverse Settling”, the flotation process consists in injecting pressurized gas at the bottom of the stainless steel vessel, in order to create an upward flow of fine bubbles. Various flotation gases can be used, such as Nitrogen (N_2), Oxygen (O_2), Carbon Dioxide (CO_2), Argon (Ar), or even air. Because of oxidation concern, inert gases such as Nitrogen or Argon are usually preferred, although they are also more expensive. The choice of flotation gas also depends on its properties regarding the bubbles it forms. An important aspect of the flotation process is the pressure employed, between 5 and 7 bars. Above 7 bars, the efficiency of the process decreases, due to difficulties of adherence between solid particles and bubbles. This is explained by the speed of migration of bubbles, too high when working at high pressure (Allen *et al.,* 1997). The solids in suspension are carried upward in the flow of bubbles, and collected at the top of the vessel forming a foam. An important aspect of this process is the necessity to correct the turbidity of the clarified must, since flotation removes a very large portion of the solids in suspension in the must. Turbidity of musts after flotation are usually between 40 and 60 NTU, hence the necessity to return a part of the lees removed, in order to avoid nutrition deprivation. The AEB E-flot 50 which the winery is equipped with, uses Nitrogen, and previously mixes the must with Gelatin to improve the efficiency of the process. Able to clarify up to 650 hL/h, this device greatly speeds up white and rosé wine processing, allowing to perform reception, pressing, clarification and inoculation on the same day.

The addition of clarification enzymes such as Novoclair® Speed or Lafazym® CL, can substantially improve the duration and efficiency of the clarification process, promoting the hydrolysis of pectins by pectinase enzymes (usually a combination of pectin lyase, pectin esterase and polygalacturonase enzymes). Indeed, pectins are vastly present in musts, and they induce a high viscosity which retains most compounds in suspension. Negatively charged, pectins also often coat positively charged proteins and induce electrostatic repulsion between these particles (Jayana *et al.,* 2005). By breaking down pectins, these pectinase enzymes expose positively charged areas of proteins which will increase the rate of flocculation and allow a faster clarification of the must. It is important to mention that some pectinase enzymes can have a cinnamoyl esterase activity, releasing cinnamic acids (p-coumaric acid, caffeic acid, ferulic acid) from their tartaric ester (Dugelay *et al.* 1993). These cinnamic acids are an important substrate for the spoilage yeast *Brettanomyces bruxellensis* (see 8.3. Brettanomyces), resulting in the formation of volatile phenols, considered as a major off-flavour. However, most commercial enzyme preparations for both skin maceration and must clarification are deprived of cinnamoyl esterase activity to reduce the risk of microbiological spoilage.

For both Cold Sedimentation and Flotation processes, the targets of must turbidity are presented in the following Table 2.

Table 2. Turbidity targets according to the wine type target.

Wine Type Target	Turbidity Target
Sparkling White	100
Sparkling Rosé	130
Light-body White	150
Full-body White	200
Rosé	250

6. 4. White Wine Fermentation

Unless maceration on skins is allowed, fermentation in white wines can rarely be spontaneous, as the must does not remain in contact with the skins where most of the native yeasts are found. Since skin contact is significantly affecting the mouthfeel, colour, and even acidity through potassium extraction leading to tartrate's formation (Jackson, 2008), it is not always desired according to the wine style targeted by the enologist. Nevertheless, spontaneous fermentation can still be triggered or enabled by various means.

The "Pied de Cuve" method consists in harvesting a small amount of grapes before the total harvest date, usually 5 to 7 days earlier, to ensure enough time for a spontaneous fermentation to start while macerating the grapes on their skins (Lonvaud-Funel *et al.,* 2010). The aromatic quality of this spontaneously fermenting must is assessed by tasting, and if judged satisfying, it will be added with a much larger amount of must provided by the harvest, once the grapes are ready to be picked. The native yeast present at the bottom of the tank ("pied de cuve" meaning "foot of the tank" in French) will be the starter culture of yeast which will colonize the rest of the young must to perform alcoholic fermentation.

Another virtual spontaneous fermentation method consists in fermenting a white wine with skin contact, and once fermentation is detected, part of this fermenting must can be transferred to other tanks, where no skin contact was allowed, and therefore provide the required yeast which can colonize the white must. The benefits of spontaneous fermentation along with the risks it represents are discussed in Chapter "7.5. Red Wine Fermentation" as such practice is more widely employed regarding red wine production, where skin contact is a necessity.

Table 3. Commercial white wine yeasts employed at Lapostolle winery.

Brand	Commercial Name	Origin	Strains	Ethanol Tolerance
Anchor	Alchemy I	South Africa	S. cerevisiae	15.5%
	Alchemy II	South Africa	S. cerevisiae	15.5%
	VIN 7	South Africa	S. cerevisiae	14.5%
	VIN 13	South Africa	S. cerevisiae hybrid	17.0%
	VIN 2000	South Africa	S. cerevisiae hybrid	15.5%
	NT 116	South Africa	S. cerevisiae hybrid	16.0%
	Alchemy III	South Africa	S. cerevisiae	15.5%
Laffort	Zymaflore X5	France	S. cerevisiae	16.0%
	Zymaflore CH9	France	S. cerevisiae	16.0%
	Zymaflore Alpha	France	T. delbrueckii	10.0%
	Zymaflore VL1	France	S. cerevisiae	14.5%
	Zymaflore VL2	France	S. cerevisiae	15.5%
	Zymaflore VL3	France	S. cerevisiae	14.5%
	Actiflore F33	France	S. cerevisiae	16.0%
Lalvin	QA 23	Portugal	S. cerevisiae	16.0%
	Ba11	Portugal	S. cerevisiae	16.0%
Enoferm	M1	New Zealand	S. cerevisiae	16.0%
	M2	South Africa	S. cerevisiae	15.0%
Uvaferm	43	France	S. bayanus	18.0%
IOC	18-2007	France	S. bayanus	15.0%
Vitilevure	58W3	France	S. cerevisiae	14.0%
Lamothe-abiet	B2	France	S. cerevisiae	14.0%
	TXL	France	S. cerevisiae	16.0%

The vast majority of white wines at Lapostolle winery are inoculated with commercial yeasts, which list is provided in Table 3. Each yeast present various performances regarding nutrition requirement, kinetics of fermentation, duration of the lag-phase, ideal temperature range, or also production of glycerol, conversion of aroma precursors into their aromatic analogue molecule, such as thiols, terpenes, esters. Tolerance to ethanol will be decisive according to the sugar content of the must, which determines the potential alcohol of the future wine. For instance, Laffort's Zymaflore Alpha is selected from the strain *Torulaspora delbrueckii* which can only withstand ethanol content of up to 10%vol. and therefore, a sequential inoculation with another yeast (from the strains *Saccharomyces cerevisiae* or *Saccharomyces bayanus* for example) is required in order to complete the fermentation. This sequential inoculation can produce a complex wine, with an increased production of esters by *Torulaspora delbrueckii* leading to tropical aromas, increase fruitiness of the wine (Renault *et al.,* 2015).

In concordance with the profile of wine desired, and with the grape variety to ferment, the choice of yeast can be determinant. A variety rich in thiols such as Sauvignon Blanc or Chenin Blanc would express its aromatic potential with yeasts like Anchor's "Alchemy II", Laffort's "Zymaflore X5" or "Zymaflore VL3", unless a less aromatic profile is desired, in which case a more neutral yeast like Lamothe-Abiet's "B2" or IOC's "18-2007" would be a better fit. Yeasts such as Vitilevure's "58W3" or Laffort's "Zymaflore VL1" present a high glycosidase activity, enhancing the release of aromatic terpenes present in large amounts in varieties like Muscat à Petits Grains or Gewürztraminer, and adding to the floral bouquet developed by such wines. Glycerol and polysaccharides are determinant for the mouthfeel of a wine, and high production of these compounds by the yeast can foresee the benefits of eventual barrel-ageing. For this reason, yeasts like Anchor's "VIN 2000", Laffort's "Zymaflore CH9" or Lalvin's "Ba11" can be well suited for barrel aged Chardonnay, Sémillon or Arinto. Therefore, the choice of yeast is a crucial tool for the enologist to orientate the profile of wine to be produced, and it is important to properly associate yeast and grape variety based on production target.

As I worked at Lapostolle winery for a short time only, before moving on to my position of Assistant Winemaker at Clos Apalta winery, I only briefly took part in white winemaking, and therefore I will not detail post-fermentation processes in this work. Nevertheless, I will describe in this work the must analysis (see Table 4 below) and the fermentation curve (see Figure 7 below) of a wine made from Sauvignon Blanc cultivar.

Table 4. Must analysis for Sauvignon Blanc from plot 918.

Code: SB 19/269 - Plot 918	
Volume (L)	**12,000**
Potential Alcohol (%vol.)	13.71
Temperature (°C)	20.6
Density	1092
TA (g/L tartaric acid)	6.12
pH	3.34
YAN (mg/L)	68.29

Planted at the density of 6666 plant/ha onto the rootstock 1103P in a colluvial soil with 10% clay, the Single Guyot cane-pruned, VSP trained Sauvignon Blanc grapevines were harvested by machine on the 27/02/2019. Stored for 24 hours in a 7°C cold room, the grapes were

destemmed by the Vaslin-Bucher Delta Oscillys® (vibrating tangential destemmer) and directly transferred to the press (Vaslin-Bucher® XPlus 50). A sparkling cycle was preferred to preserve thiols precursors from oxidation, and Lafazym® CL clarification enzymes were added, at the maximum recommended dosage of 2 g/hL. Clarification was expected to be difficult, based on previous years' observations, so a concentrated pectinase aid was added, Lafase® Boost, at 1 mL/hL. After 36h of cold sedimentation at 10℃, the turbidity reached 180 NTU and the decision to rack the wine from its lees was taken. After a slow temperature increase until 15℃, the clear Sauvignon Blanc must was inoculated with the South African yeast from Anchor, "VIN13", known for its outstanding performances, low volatile acidity or hydrogen sulfide production, low nutrition requirements and the high production of tropical thiols and esters. Throughout fermentation, temperature was maintained between 15℃ and 16℃, to ensure slow development of delicate and complex aromas. Organic nutrition (Fermaid® K at 20 g/hL) was added at the density of 1072 and DAP (di-ammonium phosphate) was added at 15 g/hL at the density of 1040.

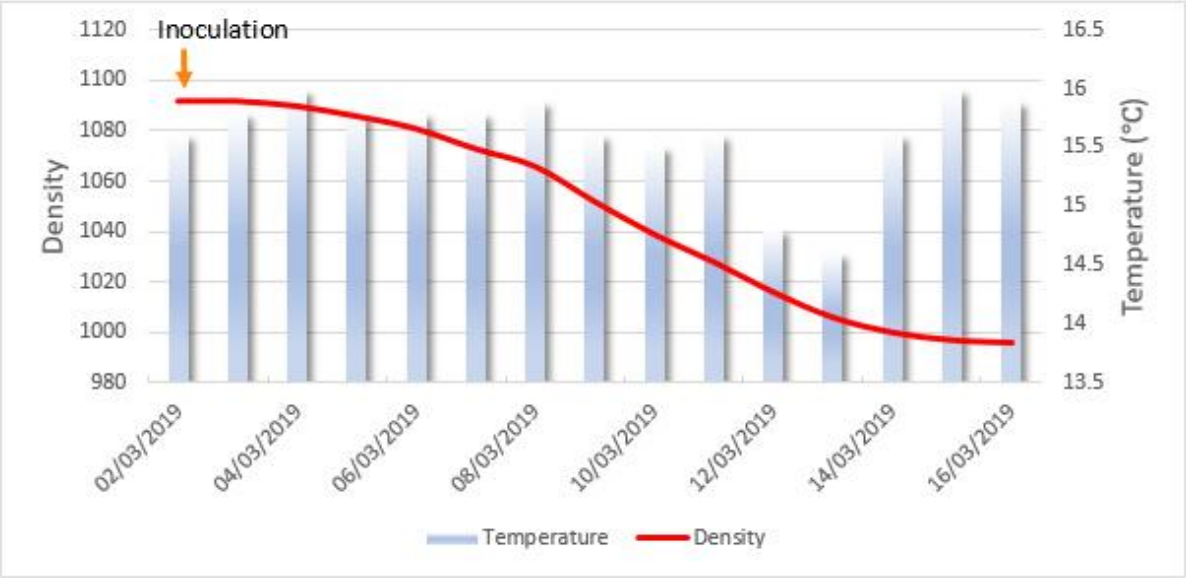

Figure 7. Fermentation curve of Sauvignon Blanc from plot 918 in tank A-29, inoculated with Anchor's Alchemy II.

The wine was protected from oxidation all through fermentation, and rapidly filtered and bottled once fermentation was complete. This Sauvignon Blanc wine offered a complex, floral and tropical nose complemented by a fresh mouthfeel, and a long lasting taste of citrus and passion fruit with a medium body and a medium to high acidity. The daily density and temperature monitoring can be found on Figure 7 above, as well as the chemical analysis of the Sauvignon Blanc wine at the end of alcoholic fermentation, on Table 5 below.

Table 5. Chemical analysis of SB 19/269 wine after fermentation completion.

Alcohol (%vol.)	13.6
Residual Sugar (g/L)	3.1
Titrable Acidity (g/L)	5.89
pH	3.42
Volatile Acidity (g/L)	0.3
Free SO2 (mg/L)	15.38
Total SO2 (mg/L)	51.22

7. Red Wine Production at Clos Apalta

7. 1. Reception

As the winery focuses on premium wines and also tries to limit its environmental impact, the clusters are solely hand-picked, and they arrive from the vineyard collected in small containers. Manipulated with precautions regarding damages to the berries and effusion of must which would get oxidized, the harvest is transported in 10 kg boxes to avoid evitable crushing. Due to the proximity between the vineyards and the winery, the boxes are stacked on the trailer of a truck, and simply covered by a black permeable clothe, but they are not refrigerated (once again, for environmental concern). Instead, they are rarely stored but processed the same day as they are picked, whenever it is possible.

Presented in Figure 8 below, the reception line starts by a vibrating table from the manufacturer Vaslin-Bucher, which efficiently removes a large part of the MOG (Material Other than Grapes) such as pieces of leaves, dust, insects by shaking the clusters at high frequency. The table ends above a small hopper, which regulates the flow of clusters arriving on the 2.80m high elevator conveyor belt, leading above the destemmer. The winery is equipped with the Socma “Le Cube” destemmer, using plastic forks and high frequency vibration to separate berries and stems under the influence of inertia. The destemmer is followed by the “Viniclean” equipment from Socma, composed of rollers, spaced of 3cm to allow berries to fall between the moving parts, while carrying the stems to the rejection conveyor belt, leading to a bin. The “Viniclean” device also features another vibrating table coupled with a filter to remove dried berries, and an extraction of juice. The sane berries will be carried to a large conveyor belt supplemented by LED lights, which compose the Sorting Table where a team of 8 trained staff

are removing any berry that doesn't qualify for premium wine's production, such as broken or sunburnt berries.

A final elevator will lead the selected berries to a movable stainless steel trolley, which can hold up to 350 kg of grapes. Once full, the trolley (equipped with wheels) will allow to transport the grapes to the fermentation room, along the platform and finally, above the opened vat where grapes will be discarded through the trolley's "butterfly" valve.

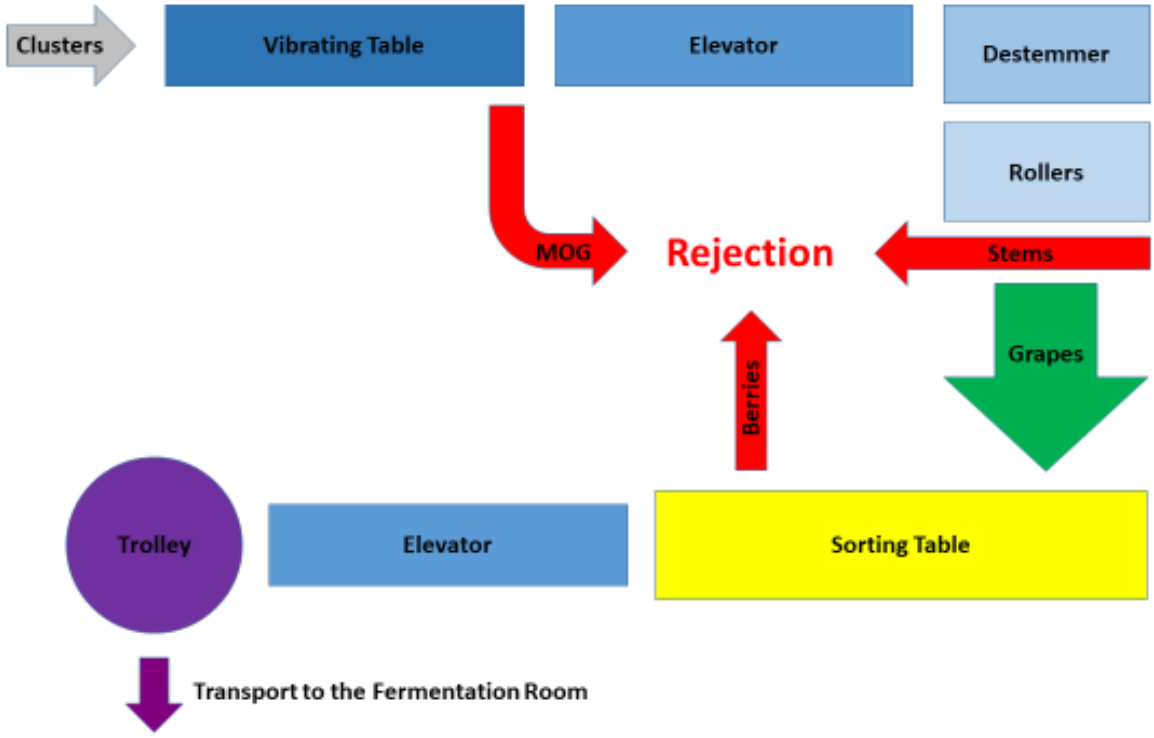

Figure 8. Organigram of the reception line, including steps of removal of rejected matter.

7. 2. Protocol of Estimation at Reception

Due to the lack of a scale large enough for a pallet or a trailer, the entire protocol of red grapes reception is based on estimations of weight. On arrival, boxes are unloaded and placed randomly on pallets, each one containing 40 boxes. A pallet from each truck is randomly selected and each box it holds will be weighted one by one, in order to calculate the average weight of a box. This average weight is influenced by the grape variety, the plot where the grapes come from, with variation in plant vigor, dehydration of berries, or simply by the picker.

Indeed, the weight of a box can largely vary, from as low as 6kg in case of severe dehydration of the berries due to the heatwave the vineyard suffered, until as high as 13kg per box, in case of healthy grapes and bad appreciation of weight by the picker. The average weight of a box usually lays between 9kg and 11kg, and although it is evaluated for one pallet only, it will be used as an estimation to calculate the total weight of grapes delivered by the truck. This operation will be repeated for each truck, and the average weights will be averaged one more time, in order to obtain an estimate of the weight of a box for the entire plot that has been picked.

At the beginning of grape's processing through the reception line (see Figure 24), another estimate will come into play. The first pallet, randomly selected, will be unloaded, each box one by one, at the start of the reception line, and once the entire pallet has been processed, the machines will be stopped for a few minutes. This short break allows the team to collect all the materials discarded by the reception line, such as Material Other than Grapes (discarded mostly by the vibrating table), stems and dried berries (discarded by the destemmer) or broken / sunburnt berries (discarded by the hand selection team). These discarded materials will be weighted, and the ratio between discarded material and initial material will be used as an estimate of rejection rate of the reception line for the entire plot.

The example used in Table 6 below, to illustrate this protocol based on estimation is the following:

- Estimate of the average weight per box W(box) = 10 kg
- Number of boxes per pallet N(box/pallet) = 40
- Estimate of the total weight of the pallet W(pallet) = 10 x 40 = 400 kg
- Number of pallets delivered from this particular plot N(pallet) = 25
- Total weight of rejection W(discarded) = 60 kg

Based on calculations presented in Table 6, we will consider for the entire plot processed that the rejection rate from the reception line is 15%, and this estimation will allow to estimate the total weight of grapes received and transferred for vinification. After reception, we now estimate for the rest of the vinification process of this particular plot, that 15% of the 10 tons received were rejected, therefore we placed 8500kg of red grapes in the fermentation vessel (see Table 6).

Another estimation, based on previous years' observations, concerns the expected conversion between weight of grapes contained in a tank, and the resulting volume of must, or volume of wine after pressing. This estimated conversion rate varies between wineries, usually between

70% and 75%. Some winemakers evaluate this conversion rate to be 70% for machine harvested grapes (higher content of MOG) and 75% for hand-picked and destemmed grapes. Here, at Clos Apalta, the conversion rate employed is 73% between weight of grapes and volume of liquid. The following additions of maceration enzyme preparation, or SO_2 will be calculated based on this estimation, either of weight or volume.

The maceration enzyme preparation used at Clos Apalta is Lallzyme® Cuvée Rouge from Lallemand, a preparation of pectinase and β-glucanase enzymes that enhance extraction of aromatic precursors and polysaccharides from grape berry cells walls, and improves the efficiency of pre-fermentative maceration. The dosage of Lallzyme® Cuvée Rouge recommended by the manufacturer is 20 g/ton. In our example, we received 8.5 tons which will require the addition of 170 g of Lallzyme® Cuvée Rouge. Once dissolved in 10 times its weight of water (1.7L), the enzyme preparation is added slowly, in 10 different additions of 170 mL each, throughout the reception of grapes to optimize the distribution of enzymes through the tank.

The SO_2 is adjusted to 4 g/hL, using a solution of SO_2 concentrated at 5%. The calculation of the necessary volume of SO_2 integrates the dilution factor of our 5% SO_2 solution, which corresponds to a dilution of 20 times. Following the same logic as the addition of maceration enzymes, the SO_2 needs to be added throughout the filling of the tank, in 10 different additions of 496.4 mL, for efficiency of protection and homogeneity purpose.

Since the estimation of the final weight / volume is known at this point of the reception process, it is possible to operate adjustments to the mass of macerating red grapes. The berries are transported mostly uncrushed between reception line and wooden vat in a 350 kg stainless-steel container equipped with 4 wheels. Once unloaded in the 75hL wooden vat, berries placed at the bottom of the vat are crushed by the weight of the berries on top, and some must is released. This allows the next step of the reception process to be performed. Referred to as “Saignée” (from the French term meaning “bleeding”), it consists in removing from the macerating mass of grapes a portion of its must. This “Saignée” will result in a decrease of the volume of must, thus increasing the ratio skin / must. The aim of this practice is to intensify the extraction of phenolic and aromatic compounds from the skin, into a smaller volume of must. Usually performed on red grapes, it allows to concentrate the resulting wine in colour, aroma and mouthfeel, intensifying its organoleptic characteristics although it also implies a diminution in the final volume of this particular red wine produced. Since the must extracted already underwent a short skin maceration, it is lightly colored and can be used for rosé wine production, to optimize the profitability of the winery and diversify its range of products. Here,

at Clos Apalta, only premium red wines are produced so the large volumes of rosé musts, protected by SO_2 and blanketed by CO_2, are rapidly transported to Lapostolle winery, where they will be blended into the only rosé wine produced by the company. The volume of the Saignée drained from each wooden vat lays between 15% (for Cabernet Sauvignon) and 25% (for Carménère) of the estimated total volume, as expressed below, according to the previous example of calculations.

Table 6. Estimations of grapes selected for fermentation, volume and must and subsequent additions of enzymes, SO_2 and volume of the Saignée.

Estimation of harvest	**Denotation**	**Value**	**Obtention**
Weight per box (kg)	W(box)	10	
Number of boxes per pallet	N(box)	40	
Weight per pallet (kg)	W(pallet)	400	*W(box) x N(box)*
Number of pallets	N(pallet)	25	
Total weight per plot (kg)	W(total)	10000	*W(pallet) x N(pallet)*
Estimation of grape sorting			
Weight discarded per pallet	W(discard)	60	
Percentage of discarded material	P%(discard)	15	*W(discard) / W(pallet) x 100*
Estimation of tank's content			
Weight of selected grapes (kg)	W(tank)	8500	*W(total) - P%(discard) x W(total)*
Volume of must (L)	V(tank)	6205	*W(tank) x 73%*
Removal of the Saignée			
Volume of Saignée (L)	V(saignée)	930.75	*V(tank) x 15%*
Addition of maceration enzymes and SO2			
Volume of maceration enzymes (g)	V(enzyme)	170	*W(tank)/1000 x 20 g/ton*
Volume of SO2 added (L)	V(SO2)	4.964	*V(tank)/100 x 4g/hL x 20*

As most of the SO_2 added directly into the vat flows away with the Free run juice drained for the Saignée (Ribéreau-Gayon *et al.,* 2006b), it is important to monitor the Free and Total SO_2 levels of both vat and Saignée. The additions of SO_2 are spread over the entire reception duration, to buffer with this consequence of the Saignée. A direct and early addition of the entire SO_2 amount, calculated for the whole volume, would result in a negligible amount in the vat, while the Saignée would contain an excessive quantity of SO_2, probably above the legal limits.

Once the entire volume of grapes has been received and transported to the wooden vat, the Free and Total SO_2 levels are measured in the Laboratory of the winery, and adjusted if necessary. The maceration enzymes do not need to be adjusted since they are calculated per ton, according to the weight of grapes received, and not to the volume of must obtained. At this point, the vat is flushed with CO_2 to protect the must from oxidation, and the pre-fermentative cold maceration is allowed to continue, under attentive care, for the next 3 days.

7. 3. Cold Maceration of Red Grapes

Mostly used on red grapes, although many winemakers decide to apply this method to some white varieties, a pre-fermentative cold maceration (also called “cold soak”) after crushing of the berries allows an increase in extraction of phenolic and aromatic compounds present in grape’s skin. Amongst them, the diffusion of phenolic compounds and particularly anthocyanins (predominantly responsible for the colour of red wines) from the skin to the must is favored by the absence of ethanol, due to the polarity and high solubility of anthocyanins in aqueous medium. Keeping the crushed red berries at cool temperature in order to limit the metabolic activity of wild yeasts present on the skin, and thus avoiding a start of fermentation, this practice aims at increasing colour intensity and stability (Boulton*,* 2001), while extracting aromatic precursors that will later add to the overall organoleptic character of the wine. Low temperatures (between 10 and 15℃) and the presence of SO_2 ensure the prevention of yeast activity, allowing enough time for an efficient extraction of those compounds, usually between 2 and 10 days for red grapes (Aleixandre-Tudo & du Toit, 2018).

Refrigeration can be achieved by various methods, where the most commonly encountered at industrial scale is the use of stainless steel tanks equipped with a jacket where cool water runs, or the use of heat exchangers submerged into the must in either wood, cement or stainless steel tanks. Previous cooling of the grapes, or addition of Dry Ice (solid carbon dioxide) can also be used, but their effect, although variable in extraction performances, is less adapted to long maceration time. Cryomaceration using Dry Ice induces a fast cooling, resulting in a temperature shock that inactivates Polyphenoloxidase enzymes, responsible for browning of the must. Furthermore, the sublimation of Dry Ice saturates the headspace of the tank with carbon dioxide, offering a supplementary protection from oxidation (Heredia *et al.,* 2010). Even though the energy demand of a cold pre-fermentative maceration isn’t negligible, the best results are achieved with a combination of these refrigeration techniques.

It appears that the early diffusion of anthocyanins in the must, due to its polarity and thus, its high solubility in water, doesn't alone explain colour intensity since it is quite an unstable and reactive compound, which monomeric concentration strongly decreases during the winemaking process. Indeed, phenomenon of co-pigmentation (metal complexation, self-association or influence of colorless cofactors such as phenolic acids or flavonols) strongly influences colour intensity and hue, even at consistent anthocyanins concentration (Boulton, 2001). Extraction of phenolic compounds such as proanthocyanidins (tannins) is slower due to a lower solubility in aqueous medium, but also play an important role on the final colour of a wine, since they form with anthocyanins the more stable polymeric pigments under the action of oxygen or acetaldehyde (Casassa & Harbertson, 2014). The presence of SO_2 as well as the cultivar show strong variations in the final concentration of phenolic compounds extracted (Casassa *et al.,* 2016). Therefore, the duration of pre-fermentative maceration has a strong influence on the final colour of a wine, since various compounds are involved, and each compound follows different kinetics of extraction. Anthocyanins reach a maximum of extraction after 5 days, while proanthocyanidins will continue being extracted and therefore allowing the formation of polymeric pigments later in the maceration process. After the fifth day of maceration, a decrease of monomeric anthocyanins is observed, attributed to the formation of anthocyanin-derived pigment, but also to a phenomenon of re-adsorption (Casassa & Harbertson, 2014). An optimal extraction of phenolic compounds during cold maceration seems to be after 10 days at low temperatures (4 - 8℃) but this duration is not necessarily adapted to large production, and needs to take into consideration the available tank space and energy consumption restrictions (Aleixandre-Tudo & du Toit, 2018).

Regarding the volatile composition of the wine produced by pre-fermentative cold maceration, a high variability in extraction of aromatic compound precursors is observed between the refrigeration methods employed (Minhea *et al.,* 2015). No significant difference was found between 3 and 6 days of cold maceration, so it appears that long maceration time doesn't improve the aromatic composition of the wine. The choice of refrigeration and cold maceration method, however, strongly influences the aromatic compounds extracted (esters, terpenes, alcohols, thiols, norisoprenoids, lactones). Furthermore, the application of pump-over and punch down (see 7.4. Cap Management Methods) during the period of cold maceration influences the extraction of aromatic compounds. There are evidences that regular pump-over cycles increase the extraction of volatile compounds, while punch down seem to have limited influence over their extraction rate (Cai *et al.,* 2014). However, pump-over and punch down promote the extraction of phenolic compounds, not only during cold maceration but through the entire maceration process, by diffusion and mechanical cell walls degradation, as such compounds are located in the berries' skin (Sacchi *et al.,* 2005).

The use of commercial maceration enzymes (pectinase, cellulose, hemicellulose, β-glycosidase) which degrades grape skin's cell walls and allows a faster release of phenolic and aromatic compounds, helps reducing the length of maceration (Ortega-Heras *et al.,* 2012). It is important to mention the necessity to choose maceration enzymes deprived of cinnamoyl esterase activity, since they would increase the concentration of hydroxycinnamic acid in the must (Tubia *et al.,* 2018), substrate for the spoilage yeast *Brettanomyces bruxellensis* (see 8.3. Brettanomyces).

Therefore, a comprehensive combination of refrigeration techniques with adapted cap management and addition of maceration enzymes can allow the winemaker to optimize the extraction of phenolic and aromatic compounds from the grape skins, while complying with logistic and energetic requirements of the winery.

7. 4. Cap Management Methods

Several techniques have been developed in order to increase polyphenol extraction (anthocyanins and tannins amongst other compounds) during maceration, period of the winemaking process that encompasses pre-fermentative cold maceration, maceration during alcoholic fermentation and post-fermentative extended maceration. These methods, regrouped under the term "cap management", aim at increasing extraction of compounds from the skins, phenolic and aromatic, but also at regulating and homogenizing the temperature through the volume of must or fermenting wine, homogenizing the distribution of yeast cells which improves their activity, and limiting microbiological spoilage (Sacchi *et al.,* 2005). During alcoholic fermentation, both heat and carbon dioxide are produced by yeast, which have an effect on solids in the medium. The carbon dioxide produced by yeast forms a flow of gas bubble that raises through the medium and carries the solids in suspension upward, resulting in the formation of a solid cap at the surface of the tank, composed mainly of grape skins and seeds. An intense microbiological activity in this cap results in a large heat production (Guerrini *et al.,* 2016). A common issue encountered at this point is the partial drying of the solids pushed at the surface of the tank, and the development and aerobic metabolic activity of bacteria such as acetic acid bacteria.

To facilitate the extraction of colour and aromas from grape's solids, and reduce microbiological spoilage's risk, breaking this cap is essential. It can be performed by a mechanical action called "punch down" or "pigeage", which consists in using a tool (a wooden

or metallic blade with a flat platform at its end) to push part of the cap back into the liquid phase, and disrupt eventual compaction areas (Sacchi *et al.,* 2005). Nowadays, modern technology allows an automation of this process, by the use of hydraulic plungers, but the cost of such equipment and its maintenance represent a substantial investment for the winery (Cai *et al.,* 2014). Punching down the cap at specified time intervals allows to maintain the solid parts of the wine hydrated, well mixed into the liquid phase for a better extraction, and promote phenolic and thermic diffusion by homogenizing the medium (Boulton *et al.,* 1999). The thermal regulation effect of the punch down method is quite limited, but the frequency of daily repetition (usually between 1 and 6 times per day) can allow the enologist to control the rate of phenolic extraction, and adjust it accordingly all through maceration (Marais*,* 2003). A special care should be taken, when working with small containers, not to crush the seeds against the bottom of the tank during the process of punch down, which would result in the excessive extraction of seed tannins.

“Pumping-over”, or “Remontage” is the action of drawing juice from the bottom of the tank, pumping it to the top of the tank, and spraying it onto the cap at the surface of the must/wine. This can be performed using a pump fitted to the tank, designed for this use, or by momentarily connecting the lower valve of the tank to a pump (Sacchi *et al.,* 2005). Pump-over can include a stage of aeration or not, according to the stage of fermentation by decision of the enologist. The enrichment on the must/wine in oxygen will promote the development and the metabolic activity of yeasts, and the formation of polymeric pigment thus improving colour stability of red wines. Nevertheless, excessive oxygenation of the must can be damaging to both colour and aroma of the wine (Moenne *et al.,* 2014). Pump-over is less effective than punch down regarding colour extraction, but more effective regarding aromatic precursors extraction (Cai *et al.,* 2014). It also shows a greater diffusion of heat through the liquid mass, allowing an increase in efficiency of the heat exchanger to cool down the fermenting wine, of major importance during the early tumultuous phase of the fermentation (Guerrini *et al.,* 2016). Applied at specified intervals, adjusted by the enologist along the course of maceration and fermentation, pump-overs allow to maintain the colour and tannin extraction as well as the temperature under control (Sacchi *et al.,* 2005). The balance between colour extraction (and stabilization) and tannin extraction (astringency and mouthfeel) is determinant for the final wine’s quality, and needs careful monitoring by regular tasting and cap management strategy adjustments.

Unlike punch down and pump-over, the method of “Délestage” or “rack and return” is not usually applied on a daily basis, but punctually, repeated or not, at a specific time during fermentation. This method consists in draining the liquid phase of the fermenting wine from

the tank, transferring it into a container, and returning it onto the pomace by a strong pumping over or by a fast discharging from the temporary container (Bosso *et al.,* 2001). The enologist can take the decision to leave the fermenting wine for an extended period of time in the temporary vessel, in order to increase its temperature and promote an efficient growth and metabolic activity of the yeast. This is usually recommended if the délestage is performed in the early phase of fermentation. Extraction of colour is optimized, compared to other cap management methods, especially through the improved formation of polymeric pigments (Zoecklein *et al.,* 2009).

The tedious, time consuming and/or energy costly character of practices such as punch down, pump-over and délestage comes from the superficial location of the cap. In contact with air, and pushed up by the flow of carbon dioxide produced by yeast during fermentation, cap management is necessary. Other techniques consist in keeping the cap permanently into the liquid phase, to avoid the practices previously mentioned. The “submerged cap” method is enabled by the installation of a steel screen inside the fermentation tank to prevent the solids (skins and stems) from raising to the surface under the effect of carbon dioxide (Bosso *et al.,* 2011). Constantly maintained into the liquid phase of the fermenting wine, the solids permanently diffuse phenolic compounds, thermal diffusion is more homogeneous and cooling is more efficient since the main source of heat is located near the heat exchanger / cooling jacket of the tank. The submerged cap method substantially increases the anthocyanin extraction compared to other cap management methods (de Castilhos *et al.,* 2017) due to the constant maceration of the skins, permanently in contact with the must. The use of a Rotary Maceration Tank also allows to constantly maintain the cap inside the liquid mass. It is composed of a horizontal cylinder tank, mounted on rails and bearings, and controlled by a motor that makes it slowly rotate, thus constantly breaking down and hydrating the cap inside the tank. This device allows an intense maceration, and extraction of polyphenols, but its energetic requirements can represent an economical drawback from its usage.

Clos Apalta winery is equipped with 21 wooden vats of 75hL each, but it does not rely on the use of submerged cap methods, nor does it aim at rapid and perhaps excessive skin compounds extraction, in the case of rotary maceration especially. The protocol of Clos Apalta winery regarding pre-fermentative cold maceration along with cap management procedure is described in Table 7.

Table 7. Daily cap management protocol during pre-fermentative cold maceration.

	8:00	12:00	16:00	20:00	0:00	4:00
Pigeage	5min	No	No	5min	No	No
Remontage (No aeration)	10min	No	No	10min	No	No
Temperature	18°C					

This daily protocol is performed for 3 to 5 days after reception of the grapes, in order to increase anthocyanins and aromatic compounds extraction. Once the extraction of colour is judged sufficient, the temperature is adjusted to 20°C in order to anticipate the inoculation, or to allow native yeasts to colonize the must and start a spontaneous fermentation.

7. 5. Red Wine Fermentation

Various strains of yeasts can be involved during alcoholic fermentation, whether it happens spontaneously or subsequent to inoculation with a commercial selected strain, or even from a Pied-de-cuve method. Each strain displays a unique behavior, regarding kinetics of fermentation, biological limitations such as ethanol tolerance, and nutrients requirements. Due to its outstanding performances, the strain *Saccharomyces cerevisiae* is considered among the most effective yeast to perform and complete alcoholic fermentation (Jackson, 2008). While spontaneous fermentation benefits from a wide range of diverse yeast strains combining their action to develop complex organoleptic characteristics, especially at the beginning of fermentation since most of them have very low tolerance to ethanol (Boulton *et al.,* 1999), it is often yeasts from the *Saccharomyces* genus that can complete fermentation. One major drawback regarding the choice of the enologist to let the wine undergo spontaneous fermentation is the uncontrolled production of off-flavors. Indeed, most yeast strains naturally present on grape's skins, such as *Hanseniaspora, Candida* or *Kloeckera* strains amongst many others, tend to produce relatively high quantities of acetic acid perceived as volatile acidity (and subject to legal limitations) and hydrogen sulfide (H_2S) perceived as reductive aroma reminiscent of rotten eggs (Bell & Henschke, 2008). Even though many winemakers consider spontaneous fermentation as the unique way to express the terroir of a vineyard, since fermentation is performed by autochthone yeasts, it also features issues of consistency between vintages, frequent stuck fermentations, and long lag phase during which the must is at risk of microbiological spoilage and oxidation (König *et al.,* 2009). This long lag phase also implies that the pre-fermentative maceration is extended, in comparison to a wine rapidly

inoculated by a selected strain, and this has to be in concordance with the wine style that the winemaker aims for.

Even though the enologist of Clos Apalta allows spontaneous fermentation to happen in few tanks (with grapes from specific plots, based on previous years' observations), most of the wines are inoculated. The various yeasts employed at Clos Apalta to initiate fermentation are presented in Table 8.

Table 8. Commercial red wine yeasts employed at Clos Apalta winery.

Brand	Commercial Name	Origin	Strains	Ethanol Tolerance
Anchor	Alchemy III	South Africa	S. cerevisiae	15.5%
	Alchemy IV	South Africa	S. cerevisiae	15.5%
	NT 50	South Africa	S. cerevisiae hybrid	16.5%
	NT 116	South Africa	S. cerevisiae hybrid	16.0%
Laffort	Zymaflore F15	France	S. cerevisiae	16.0%
	Zymaflore RX60	France	S. cerevisiae	16.5%
	Zymaflore Xpure	France	S. cerevisiae	16.0%
	Actiflore F33	France	S. cerevisiae	16.0%
Lalvin	BM4X4	France	S. cerevisiae	16.0%
	Clos	Catalunya	S. cerevisiae	17.0%
Enoferm	RP15	California	S. cerevisiae	17.0%
	Syrah	France	S. cerevisiae	16.0%
Uvaferm	43	France	S. bayanus	18.0%
	BDX	France	S. cerevisiae	16.0%
IOC	R 9008	France	S. cerevisiae	16.0%

Laffort's "Zymaflore F15", selected from Bordeaux in France, is the main yeast in use at the winery, as it enhances varietal aromas with a relatively high production of glycerol, promoting the mouthfeel of full-bodied wines suitable for barrel ageing. IOC (Institut Oenologique de Champagne) commercializes the "R 9008" yeast which, according to the enologist, tends to decrease herbaceous notes imparted by methoxypyrazines and for this purpose, it is also used in the winery for some Carménère and Cabernet Sauvignon, rich in this compound. Other notable yeasts from this list include Lalvin's "Clos" which features a low colour adsorption character, suitable in case of low anthocyanin's content, or Anchor's "Alchemy III" from South Africa, which features a high production of esters, higher alcohols and damascenones to increase the aromatic intensity of the wines. Uvaferm's "43" is originated from the *Saccharomyces bayanus* genus, and benefits from an ethanol tolerance of up to 18% vol. and a fructophilic character, which make it suitable to restart a stuck fermentation.

The majority of wines at Clos Apalta are fermented by Zymaflore F15, whose performances and consistency have proven great potential for the enologist. Most grapes received and selected are transferred to a 75 hL wooden vat, either as a single plot batch, or blended between plots of complementary chemical characteristics determined in the laboratory. After the 3 to 5 days' cold maceration period discussed above, the vat is inoculated with yeast prepared as advised by the producer. Preparing the yeast consists in placing it in 37℃ water (1L/100g of yeast), allowing it to rehydrate for 20min, before adding slowly some must (1L/100g of yeast) to cool the mixture down without any temperature choc, until a maximum of 5℃ of temperature difference with the must is reached. This allows the yeast to slowly acclimate to a colder environment, with a high content of sugar, and thus avoid any thermal or osmotic choc once added to the must. To avoid a choc triggered by rapid acidity variation, and also to buffer any acidity modification in the must, the water used to rehydrate the yeast is added with 4 g/L of tartaric acid. The cycle of cap management is maintained as it was during pre-fermentative maceration, except the temperature set-point which is fixed at 20℃. It is important to mention that inoculation is performed at the beginning of a remontage in order to homogenize the must and diffuse yeast in the entire volume.

Fermentation is assessed by tasting, until CO_2 production is detected, and the protocol of cap management can be adjusted. The remontage are now performed with aeration, to provide the active yeast cells with oxygen, necessary for their multiplication and metabolism. Indeed, at an early stage of development of the yeast population, oxygen is consumed for the synthesis of sterols and unsaturated fatty acids, necessary for the production of healthy yeast cells membranes, and thus viability and ethanol tolerance of the population (Fornairon-Bonnefond *et al.,* 2002). A deficiency of oxygen during the exponential phase of yeast population development has been reported to be a potential cause of a later stuck fermentation (Malherbe *et al.,* 2007). Remontage with oxygenation also allows to release some of the CO_2 (produced by the yeast) from the must, while dissolving the necessary oxygen in the medium. The remontage cycles are adjusted to 15min, with the peristaltic pump set at 300 hL/hour, thus determining the volume of wine pumped over at each remontage to 75 hL, the entire volume of the vat. At each cycle, the entire volume of fermenting wine will be oxygenated, discarded of its excess of CO_2 and the temperature will be homogenized.

Along the course of alcoholic fermentation, 2 délestages are performed, at the densities of 1075 and 1040. The entire liquid volume of the vat is transferred to a storage vat, through a stage of aeration similar to the aeration occurring during the remontage. The must will stay there for 10 to 12 hours before being returned on top of the pomace left in the original vat. This process, described in Chapter "7.4. Cap Management Methods" is meant to extract large

amounts of skin compounds such as anthocyanins, tannins and aromatic precursors, and to completely disrupt the structure of the cap and enhance the efficiency of later pigeages and remontages.

Temperature control could be automatic, since the vats are fitted with a thermometer and an electronic programmer that trigger valves, controlling the hot and cold water circuit that can run through the stainless steel serpentine in the center of the vat. This automatic system has proved in the past to be unreliable, due to the distance between the serpentine and the thermometer, and also due to the lack of convection movement within the volume of must. In case of white wines, pressed and separated from the pomace, convection movement in the liquid volume would allow a better thermal diffusion and a better homogeneity. However, in red wine fermentation, the amount of solids present in the vat doesn't enable efficient convection and the thermal dispersion is very irregular through the volume of must. Consequently, if the serpentine is fed with cold water (at 7℃) the temperature of the must at the center of the vat will be strongly affected by the decrease in temperature, often excessively, while the must at the circumference of the vat won't be affected by the cooling. To buffer this effect, only short sequences of heat / cold are performed, between 5 and 10 minutes long, spread along the day. Furthermore, cooling / heating the fermenting must is recommended during the remontage, as the temperature homogenization will allow a more accurate monitoring of the temperature variation induced through the serpentine.

In order to ensure thermal stability for the yeast, the temperature is slowly allowed to increase, at the maximum rate of 2℃ per day, from 20℃ at the stage of inoculation to 28℃, ideally reached at the end of the exponential phase and maintained for the stationary phase of the yeast's population timeframe. These relatively low temperatures allow a slow fermentation, which results in an increase of aromatic intensity of the wines, attributed to a longer timeframe for the enzymatic action of glycosidase, esterase and other yeast-related metabolisms involved in the liberation of aromatic compounds present in the must under the form of aromatic precursors (Sablayrolles, 2009).

Table 9. Daily cap management protocol during alcoholic fermentation.

	8:00	**12:00**	**16:00**	**20:00**	**0:00**	**4:00**
Pigeage	5min	5min	5min	5min	5min	5min
Remontage (with aeration)	15min	No	15min	15min	No	15min
Temperature	20 - 30℃					

According to the evolution of astringency perception's in the wine, solely relying on frequent tasting, the extraction of tannins and their effect on the mouthfeel can be evaluated. In case of an early excessive astringency, usually perceived around the final days of fermentation (due to the duration of maceration and to the apolarity of proanthocyanidins, preferentially extracted in ethanol than water), the temperature can be adjusted to 32℃ and maintained for 2 to 5 days in order to stimulate tannin polymerization and smoothen the astringency and the overall mouthfeel. Nevertheless, here at Clos Apalta winery, temperature is usually risen to 28℃ at the end of fermentation to support the metabolism of yeast cells and ensure their ability to complete the fermentation, even in a hostile environment due to ethanol toxicity. This is matter of debate as there are evidences showing the synergic correlation between high temperatures and ethanol toxicity for the yeast cells, justifying the benefits of maintaining temperature around 25℃ for the end of alcoholic fermentation (Malherbe *et al.,* 2007).

At this stage, the remaining yeast cells' activity significantly decreases, and production of CO_2 is very low. Without the continuous upward flow of carbon dioxide produced all through fermentation, yeast cells tend to settle at the bottom of the tank, leaving a small amount of residual sugar in the wine. For both microbiological stability and organoleptic reasons, a complete fermentation is desired, resulting in a dry wine (below 3.5 g/L of residual sugar). Therefore, returning the settling yeast cells into suspension is necessary for them to complete the consumption of sugars present in the wine. This can be reached by a rapid remontage, but in case of excessive astringency, it is not recommended to pump the wine over the cap as it is otherwise done. In this situation, the remontage is performed between the lower valve of the vat, and the valve positioned at 50cm from the bottom of the vat. Both types of remontage include a step of aeration to promote phenolic polymerization and result in softening the tannins and increasing colour intensity. Both practices also allow yeast cells to be returned into suspension in the wine where they will eventually enter in contact with residual sugars and metabolize it.

Alcoholic fermentation is considered completed when the concentration in residual sugar reaches values below 3.5 g/L. This threshold value ensures the scarcity of sugar in the volume of wine, which otherwise would be considered a source of microbiological instability regarding metabolisms using sugar as a substrate. This is particularly relevant for the activity of Lactic Acid Bacteria which would metabolize sugar and produce acetic acid, detrimental to wine aroma (König *et al.,* 2009).

7. 6. Post-fermentative Maceration and Pressing

Once alcoholic fermentation is considered complete, after verification of residual sugar in the laboratory, a post-fermentative maceration is allowed for a duration which is variable between each wine according to tannins extraction and polymerization. This step of the vinification process consists in maintaining the wine at 28-32℃ for a period between 7 and 14 days (according to the evolution of the wine assessed by daily tasting), thus promoting the formation of tannin polymers which improves the mouthfeel of the wine by lowering the astringency, mainly attributed to the contribution of monomeric and oligomeric tannins. Nevertheless, there is evidence that the tannins extracted by such extended maceration originate mainly from the seeds, thus increasing astringency overtime (Casassa *et al.,* 2012).

Once the mouthfeel is satisfying, the wine is ready to be transferred to oak barrels in the room below, “Primero año”, and the remaining pomace is ready to be transferred to the press. First of all, the portion of the wine that runs out naturally without mechanical action, called “Free run wine” in opposition to the “Press wine”, is allowed to flow from the lower valve to a stainless steel vessel, connected through a hose to the valve embedded in the ground, as discussed in Chapter “4. Winery Description” above. This valve leads to the “Primero año” room below, where the other end of the valve is itself connected to another hose, which ends by a tap. This system enables the filling of barrels in the room below the Fermentation room, without the use of any pump, simply relying on the gravity. By opening and closing the tap at the end of the last hose, it is possible to fill each barrel, one by one, with the Free run wine that flows from the vat.

When the flow of Free run wine becomes weak, the door of the vat is opened, in order to proceed to the next step. The pomace forms a flat layer, inside of the vat, and covers the outlet of each valve. It is then necessary to use a shovel to remove the pomace from the area around the valve outlet, and throw it at the back of the vat. The name of this step, “Meia Luna” (or “Half moon” in English) refers to the desired shape of the aperture around the valves, through the layer of pomace. The aim of this step is to free the valve from pomace, and allow more Free run wine to slowly drain out of the vat. Indeed, the Free run wine is considered of higher quality than the Press wine, as it is lower in monomeric tannins, less bitter, less oxidized (Ribéreau-Gayon *et al.,* 2006c). Consequently, it is interesting to maintain Free run and Press wines separated through the stage of ageing, as it will enable the elaboration of a finer final blend.

After 12 hours of draining, the pomace can be removed from the vat and transferred into the press. As a member of staff needs to enter the vat in order to shovel the pomace out of the door, it is absolutely mandatory to previously place a fan on top of the vat which door needs to remain opened. This safety measure recycles the air from within the vat, as it is almost saturated with CO_2 and entering the vat would be a safety hazard for the employee who will dig out the pomace, at risk of asphyxia and suffocation. Every year, there are casualties amongst winery employees around the world, even employees with experience, and this ventilation safety measure is therefore mandatory, as is the presence of a second employee outside the tank or vat to ensure the person digging out the pomace does not faint, and react rapidly otherwise.

The pomace is placed in the basket of the press (Clos Apalta is equipped with a Vaslin-Bucher JLB 12 of a capacity of 12hL) and the cycle previously programed (see Figure 9) can be started. This cycle aims at applying a low pressure (0.2 bar) for the first 20min phase called Step 1. The second phase, Step 2, also lasts for 20min, and applies a pressure of 0.4 bar, considered by the enologist to extract slightly more tannins. Step 3 applies 0.8 bar for 20 more minutes. From Step 4 until the end of the cycle, the pressures applied are consequent, between 1 and 8 bar, which tends to extract more bitter monomeric phenols (Ribéreau-Gayon *et al.,* 2006c), mainly from the seeds. For this reason, the press cycle is segmented in 3 distinct parts, P1 which encompasses Steps 0 and 1, P2 which encompasses Steps 2 and 3, and P3 from Step 4 until the end. P1, P2 and P3 are transferred to barrels separately, once again for optimization of the final blend. It is important to mention that Press wines are often transferred to second generation barrels, from the previous year, as they will usually be used for the second label produced by the winery, hence a smaller investment for these wines in terms of oak barrels' costs. The large amount of tannins extracted by the pressing of the pomace also justifies this enological decision.

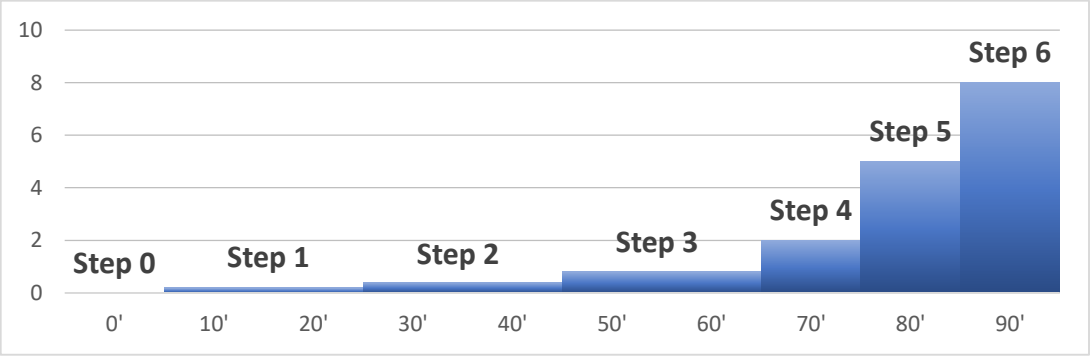

Figure 9. Histogram of the pressure cycle applied on the pomace, in bar (vertical axis) over time in minutes (horizontal axis)

7. 7. Barrel Fermentation

Most wines are fermented in 75 hL wooden vats, as previously mentioned, but in some occurrences, the yield of some plots can be higher than the amount fitting in a vat. In this case, the excess of grapes is placed in oak barrels where they will undergo the entire alcoholic fermentation.

This process requires to previously open the top part of the barrel (which is positioned vertically) using a hammer and a tool called “hoop driver”, to remove the first 2 “hoops” which are the metal rings that tightly hold the staves together. After removing the head-hoop and the second hoop, it is necessary to loosen the first middle hoop, in order to loosen and remove the entire board of flat stave that forms the head of the barrel, as a whole since the staves are fixed together. Replacing the hoops and tightening back the center of the barrel is necessary before filling it with grapes.

Once full, it is recommended to draw the Saignée of 15% as in large vats, to lower the level of liquid inside of the barrel before proceeding to the next step, the closure. This can be achieved by loosening the middle hoop again, for a short time necessary to carefully replace the head of the barrel, using a hammer. This step is tedious and delicate, as the head needs to fit perfectly in the circular cavity designed for it. If the level of liquid is too high inside the barrel during this operation, some must will leak between the staves (see right picture in Figure 10 below), and this will complicate the replacement of the middle hoop, hence the utility of previously performing a Saignée. Once the head is back in its original position, the middle hoop can be tightened again, the second hoop can be repositioned and finally, the head-hoop can be tightened to close the barrel completely.

Figure 10. Pictures of the empty barrel (left) with the head-hoop, second hoop and middle hoop in place and the barrel's head separated, the barrel filled with Cabernet Sauvignon grapes (center) and the barrel closed back using the hammer and hoop driver displayed.

Unlike in a large container, cap management is imprecise, since it consists in closing the barrel and making it roll back and forth in order to simulate cap irrigation and breaking down, or punch-downs using a small metallic tool (originally aimed at performing "bâtonnage"). Although oxygen is slowly diffused through the pores of the wood, aeration required by the yeast is only possible through micro-oxygenation, which is applied by the mean of a spurge connected to an oxygen cylinder, releasing oxygen at 1 bar for 60 seconds at the density 1075, 30 seconds at 1040, and 10 seconds at 1010 (Gómez-Plaza & Cano-López, 2011). Although barrel fermentation doesn't allow temperature control, the resulting wines differ greatly from the wines fermented in vats, originated from the same plot. Barrel fermented wines tends to have a higher colour intensity, due to copigmentation promoted by the permanent oxygen uptake, and well-integrated aromatic notes of oak and toast. The difference is depth of colour and hue is presented in the picture below, in Figure 11, where both wine samples have been taken after 5 days of fermentation. We can observe a large difference in colour intensity, much higher in the wine fermented in barrels (left) with darker tones than the wine fermented in the vat (right). The mouthfeel of barrel fermented wines also tends to be richer than their wooden vat analogue. Once alcoholic fermentation has been completed and the post-fermentation maceration is considered sufficient, the barrels are drained and the free run wine is transferred to another barrels where it will undergo malolactic fermentation. The barrels are then positioned vertically, and the first 2 hoop are removed. This loosens the top part of the barrel which is also removed, in order to transfer the pomace to the vertical basket press, where the

press wine will be extracted. Once emptied, the barrels are reassembled, cleaned, sulfur is burnt inside as previously described (see 8.5. Sulfur Dioxide) and transported to Lapostolle winery, where they will be used as second generation barrels.

Figure 11. Picture comparing 2 wines sampled produced by either barrel fermentation (left) and by fermentation in a 75hL vat (right) after 5 days of fermentation.

However, the cost of such practices is high, as it constantly requires manipulations such as punch-downs and rotations (every 4h), micro-oxygenation, opening and closing of barrels, drawing of Saignée to lower the level of must in the barrel or also to prepare inoculation and nutrition. For each Saignée, it is necessary to draw the same amount of must from each barrel of a given batch, which can require extensive hours of work.

For the entire harvest, a total of 290 barrels fermented wines were produced. At the pick of harvest, Clos Apalta held 168 barrels simultaneously undergoing alcoholic fermentation and maceration of red wines. For such amount of barrels, a sequence of punch-down and rotation requires 4h for one trained and efficient worker, and is necessary every 4h, which turns out to be a 24h consistent task. Opening and closing the barrels' top after grape's reception also requires one person permanently, as it is a long and tedious task. Performing of Saignées and

addition of yeast, nutrition or acidity correction also requires multiple hours of work according to the size of the batch.

Once these labor parameters taken into account, particularly the necessity of a staff member permanently dedicated to barrel manipulations, and another staff member permanently opening and closing them after reception, and again after fermentation is completed, such practice appears to be extremely costly. Only adapted to icon wines, where the large price margin can cover the cost, barrel fermentation definitely adds value regarding communication to visitors of the winery, but whether it adds organoleptic value on the final product is uncertain. It is yet to determine if the organoleptic impact on the final wine made from this type of fermentation is significantly improved, compared to a fermentation in wooden vat followed by malolactic fermentation and ageing in brand new oak barrels. According to the enologist, wines fermented in new oak barrels display very well-integrated oak aroma, and a more stable colour than its wooden vat antagonist wine, but more research would be necessary to confirm this statement.

7. 8. Carbonic Maceration

Experiments are being conducted regarding the contribution of a small percentage of the final blend produced by Carbonic Maceration. Invented in 1934 by Michel Flanzy, Carbonic Maceration (denoted CM) consists in placing whole clusters or intact berries in a closed tank saturated with a CO_2 atmosphere. Submitted to these anaerobic conditions, the berries undergo intracellular fermentation without any intervention of yeast, but instead under the anaerobic activity of endogenous enzymes, resulting in the conversion of a small amount of sugar into ethanol, usually around 2% vol. (Tesniere & Franzy, 2011). Beside producing ethanol, this intracellular metabolic phenomenon consumes a large amount of malic acid, and has a strong influence of extraction of phenolic and aromatic compounds (Pace *et al.,* 2014).

As berries undergo CM and break, or under the weight of other berries, some must is released and yeast-induced alcoholic fermentation occurs at the bottom of the tank. After 7 days of CM or more, according to temperature, grape maturity or simply by target of production, berries are crushed, pressed, and yeast can complete alcoholic fermentation without maceration of the skins (Tesniere & Franzy, 2011). Wines resulting from CM show low content of monomeric anthocyanins, but a high content of pyranoanthocyanins and anthocyanin-derived pigments, maintaining a sufficient although reduced colour intensity, compared to regular maceration wines (Chinnici *et al.,* 2009). The hue of these wines tends to show more orange character,

due to the presence of pyranoanthocyanins. Content of monomeric catechin and proanthocyanidins is increased with CM, but since the alcoholic fermentation is performed off the skins, the final polyphenol content of the wine is lower than in regular fermentation in the presence of skins. Also, since the anthocyanins content is lower, the formation of polymeric pigment is negatively affected, and this results in wines with limited ageing potential (Sun *et al.,* 2001). The volatile composition of wine that underwent CM is strongly modified, with an increase in fruity character, suitable for a youthful wine. Even though its colour is lowered and its hue is shifted toward brick-red, the overall organoleptic character of CM wines is pleasant with low astringency and a fruity aroma, often described with notes of "banana" or "bubblegum" (Spranger *et al.,* 2004). Nowadays, several methods are employed in wineries to induce CM. According to Tesniere & Franzy, 2011, we can distinguish:

- The original method of carbonic maceration where CO_2 is injected in the closed tank, either under gaseous form or released from the sublimation of dry ice, and the intact berries are submitted to the anaerobic fermentation for a specified period of time.

- A method which consists in placing berries or entire clusters in the closed tank, and under the weight of this mass of grapes, some berries at the bottom will be crushed, some must will be released and a spontaneous fermentation will occur. This will produce some CO_2 which will fill part of the atmosphere of the tank, and induce CM on intact berries.

- A partial CM can be achieved by placing some entire clusters in a tank filled with crushed berries, where alcoholic fermentation produces CO_2 which will induce CM in the intact berries from the whole clusters. The percentage of whole cluster will determine the intensity of the CM character on the final wine.

At Clos Apalta, as an experimental project, this can be conducted in small stainless steel tanks usually used to transport to Lapostolle winery the rosé musts extracted from the Saignées. This phenomenon can be triggered added with Dry Ice to saturate the volume with CO_2 and induce a complete CM, or by adding a certain percentage of whole bunches to a tank in order to obtain a partial CM. These trials, however, are always performed with clusters of high phenolic maturity, especially the stems, since green stems would affect the organoleptic characteristics of the wine by adding herbaceous notes, and astringent mouthfeel due to their high concentration in monomeric tannins (Souquet *et al.,* 2000).

7. 9. Yeast Nutrition Requirements

In order to optimize the performances of the fermenting yeast, it is necessary to provide it with an adapted nutrition. Indeed, in case of nutritional depletion or deficit of some specific substrates, the yeast is considered “stressed” and this results in the production of acetic acid and the release of various amounts of hydrogen sulfides (H_2S) which can later promote the formation of mercaptans and its reductive off-flavour (Bell & Henschke, 2008). The main nutrient, indispensable to its metabolism, is Nitrogen, which can be provided under various forms. The main sources of Nitrogen available to the yeast is ammonia (NH_3) and amino-acids present in the must. Together, these sources of Nitrogen can be measured as Yeast Assimilable Nitrogen, or YAN, in mg/L. To provide the yeast with an adapted amount of nutrients to perform the alcoholic fermentation, the YAN needs to be proportional to the sugar content of the must, such as for each 1 g/L of sugar, 0.75 mg/L of YAN are necessary (Mendes-Ferreira *et al.,* 2004). In such conditions, the yeast cells will be supplied with sufficient Nitrogen to synthetize compounds required for their metabolic activity, and will be able to successfully perform alcoholic fermentation while withstanding osmotic pressure of the sweet must, and later withstanding ethanol toxicity of the wine. Normal YAN values for a safe fermentation usually lays around 140 mg N/L (Gobert *et al.,* 2019).

However, during the early phase of the yeast cells development, the YAN being too high would lead to an excessive multiplication of the yeast population, resulting in a rapid depletion of nutrients from the must, and consequently this large population of yeast would quickly become stressed. This early excessive availability of Nitrogen would lead to an increase in volatile acidity (acetic acid and ethyl acetate) and reduction aroma (hydrogen sulfides) in the fermenting must (Bell & Henschke, 2008). Therefore, it is usually admitted that, when necessary, the first nutrition aiming to adjust the YAN at the desired starting value needs to be performed when the density has decreased of 20 units from the initial must density. This also ensures that most autochthones yeasts have already stopped developing and the Nitrogen supplied will be used by the desired yeast strain (Pretorius, 2000). The second nutrition helps ensuring that the yeast population will be in optimal conditions to carry on the fermentation until its completion, since the environment will become very hostile toward yeast cells later through fermentation, mostly due to ethanol toxicity (Gobert *et al.*, 2019). The timing of the second nutrition, just like the first nutrition, is important. It needs to be added before the excessive presence of ethanol diminishes the yeast cells’ permeability, and thus its ability to uptake nutrients from the must. This corresponds to the end of the exponential phase of

yeast's development, around 1/3 of sugar depletion. According to the winemaker of Clos Apalta, the yeast population average requirement in YAN at this point of its development timeframe is about 30 mg/L, and this was the systematic target for the second nutrition, but this value is matter of debate and needs further scientific investigation.

Nutrition provided to yeast cells can be classified in 2 main categories: Mineral (or Inorganic) and Organic. Mineral nutrition, mainly di-ammonium sulphate and di-ammonium phosphate, are presented as salts, dissolvable in the must to enrich it with free ammonium, easy to uptake by the yeast (Hernandez-Orte *et al.,* 2006). Commercial brand can sell it as "DAP" (di-ammonium phosphate only) or it can also be added with thiamine (vitamin B1) to complement its nutritional value, such as Thiazote®. Both Thiazote® and di-ammonium phosphate (DAP) increase the YAN by 21% of their weight.

Organic nutrition, on the other hand, originates from autolyzed or inactivated yeast, and brings the essential free amino-acids and other sterols, unsaturated fatty acids and vitamins (thiamine, pantothenate, biotin, niacin) to the yeast population. Organic nutrition is often complemented by di-ammonium phosphate to cover most of the needs of the yeast, and its impact on the YAN of the must is variable, usually between 7% and 13% of its weight. Each nutrient it brings to the yeast has a specific role in its metabolism. Thiamine or vitamin B1, for instance, is necessary for the yeast to convert pyruvate into acetaldehyde, and later into ethanol (Jackson, 2008). Pantothenate or vitamin B5 is necessary for the yeast to complete the metabolism of Cysteine and Methionine, the 2 sulfur-containing amino acids, which it produces from hydrogen sulfides (H_2S) previously metabolized. A lack of pantothenate would lead to an accumulation of H_2S in yeast cells, released after yeast's autolysis and the apparition of its characteristic reductive notes (Jackson, 2008). Unsaturated fatty acids and sterols are required by the yeast for the development of a healthy membrane, which will determine its alcohol resistance and its permease activity, to uptake nutrients from the must (Aguilera *et al.,* 2006). Organic nutrition often comprises yeast cell hulls, or inactivated yeast cells. Their effect on the medium, besides enriching it in the nutrients previously stated, comes from their ability to adsorb medium chains fatty acids, toxic to the yeast and thus inhibitors of fermentation. This detoxification of the medium promotes easier conditions for the yeast to complete the fermentation, by limiting the hostility of the environment it will be submitted to, especially at the end of alcoholic fermentation (Lafon-Lafourcade *et al.,* 1984).

Whether the first and/or the second nutrition should be organic or inorganic is matter of debate but it eventually comes down to targets of wine style to produce (Torrea *et al.,* 2011). Organic nutrition contains large compounds such as amino-acids which uptake by the yeast would be

compromised by the excessive presence of ethanol. These amino-acids, besides being metabolic functional compounds, are also used as a source of Nitrogen for the yeast, which will lead to the formation of higher alcohol and esters, following the catabolic pathway described by Ehrlich at the beginning of the past century, often referred to as the Ehrlich pathway (Hazelwood *et al*., 2008). Therefore, the addition of organic nutrition at the beginning of fermentation promotes the fruity character of a wine.

Table 9. Nutritional additives employed at Clos Apalta winery.

Nutritional additive	Type	Nitrogen Content
Di-Ammonium Phosphate	Inorganic	21%
Thiazote®	Inorganic	21%
Optiferm®	Organic (mixed)	13%
Fermaid K®	Organic (mixed)	13%
Fermaid O®	Organic	7%

In order to facilitate the use of nutritional additives in the winery, I have processed information given by the enologist, and developed a decision-making tool called “Correction YAN”. Based on a calculation sheet, this digital tool allows the user to obtain the precise weight of inorganic and organic nutrition required in a specific must to ensure a safe fermentation. Assisted by this tool, the user needs to inform few parameters determined in the laboratory about the must and will obtain recommendations of nutrition. This assistance tool offers the choice regarding organic/inorganic repartition preferences, which will determine the necessary additions’ weight.

As presented below, “Section 1” of Figure 12 concerns information on the must, as it requires elements highlighted in blue, from which other useful characteristics are calculated. According to Considine & Frankish (2014), the sugar content in g/L can be expressed using the conversion factor from potential alcohol, determined as 16.83 g/L of sugar for each unit of potential alcohol, or the °Brix, given by the following relation to the density of the must.

$$°Brix = 182.4601 \times D^3 - 775.6821 \times D^2 + 1262.7794 \times D - 669.5622$$

Recommendations regarding the YAN are presented by “Section 2”, such as the Optimal YAN value calculated such as 1 g/L of sugar requires 0.8 mg/L of YAN (as explained above), and the Minimum YAN value expressed as 75% of the Optimal YAN. The difference between these values and the actual YAN of the must are presented as Deficit to Optimal and Minimum YAN.

From these YAN value recommendations, 2 different scenarios of nutrition are expressed in “Section 3”, the Optimal YAN and the Minimal YAN additions status. They are both calculated such as inorganic nutrition is mainly used to bring the YAN of the must up to a threshold value of YAN fixed at 150 mg/L, and the organic nutrition will contribute to increasing the YAN until the final desired value, whether it is the Optimal or the Minimum YAN value.

“Section 4” concerns the decision of the user about the additions he/she judges accurate in order to correct the YAN of the must. Both organic and inorganic nutritional decision will have an impact on the YAN, according to the Nitrogen content of the product/brand used (which needs to be informed in “Section 5”), and the tool verifies that the increase of YAN produced by the additions doesn’t exceed the Optimal YAN value. In case of an increase within the boundary of the Optimal YAN value, the YAN Increase box appears green (see Section 4, example 1). In case of an excess of YAN, the YAN Increase box appears red (see Section 4, example 2). In either situations, the total weight of necessary additions is calculated, for the volume of must informed in “Section 1”.

Finally, “Section 6” requires a decision regarding the repartition of organic / inorganic nutrition, by adjusting the desired value in the blue box, and calculates the percentages of repartition based on this decision. The calculations ensure that the second nutrition will increase the YAN of 30 mg/L, independently of the choice of repartition from the user. The density at which the first nutrition is calculated to be 20 units below the initial density, and the second nutrition is fixed at the density of 1040, as requested by the enologist. Following the percentages of repartition of the desired nutritional additions, their weight (in grams) is expressed for both first and second nutrition, allowing the user to quickly obtain the accurate amounts of products to be used.

This tool needs to be updated in case of a change in the products used in the winery, as they present variable Nitrogen content, but “Section 5” is facilitating this modification. However, it is the user’s responsibility to ensure the additions are in compliance with legal limitations, for instance 100g/hL for DAP and Thiazote®, or 40 g/hL for Optiferm® and Fermaid K®.

Section 1:

Volume (L)	Potential Alcohol	Density	Sugar (g/L)	°Brix	YAN in must (mg/L)
6,000	14	1100	235.6	23.8	100

Section 2:

Recommended YAN		Deficit to	
YAN Optimal	YAN Minimum	YAN Optimal	YAN Minimum
188	141	88	41

Section 3:

Recommended Addition	YAN Optimal (g/hL)	YAN Minimum (g/hL)
Inorganic / Mineral	30	20
Organic	20	0

Section 4:

example 1:

Addition (g/hL)	YAN (mg/L)	Total (g)
30	63	1800
20	26	1200
YAN Increase:	88	

example 2:

Addition (g/hL)	YAN (mg/L)	Total (g)
31	65	1860
20	26	1200
YAN Increase:	91	

Section 5:

Nitrogen Content of Nutrition:	
Inorganic:	21%
Organic:	13%

Section 6:

	Repartition (%)		Repartition (g)	
Density	1080	1040	1080	1040
Inorganic / Mineral	63%	37%	1129	671
Organic	75%	25%	900	300

Figure 12. Digital calculation tool called “Correction YAN” separated by Sections.

7. 10. Malolactic Fermentation

Malolactic fermentation (MLF) is an important step of the winemaking process, although it is not systematic according to the wine style to be produced. This secondary and optional fermentation can be performed by a wide range of bacteria, regrouped under the Lactic Acid Bacteria (LAB) denomination. The most notable LAB used in winemaking are *Oenococcus oeni, Lactobacillus Plantarum,* or even some species from the *Pediococcus* genus. Regarding winemaking, their metabolic activity of interest is the decarboxylation of L-malic acid into L-lactic acid, metabolism releasing carbon dioxide. The main effect of MLF is the deacidification of the wine, since malic acid is a stronger acid than lactic acid, and this results in a slight rise in pH (Bartowsky *et al.,* 2015). The consumption of L-malic acid also contributes to long-term microbial stability of the wine, as malic acid can be used as a source of carbon, if available, for spoilage organisms.

Overall, wines are perceived to be smoother and softer after MLF, which makes it a very common practice for red winemaking, or for medium and full bodied white wines. However, MLF is not necessarily regarded as an improvement but as a spoilage for the production of youthful fresh white wines where a rather high acidity is desired, for instance. Besides deacidifying the wine, MLF has a significant impact on wine's aroma and flavor, as LAB can metabolize citric acid into diacetyl, which aroma is often perceived as buttery (Bartowsky & Henschke, 2004), or produce enzymes such as glycosidase and esterase (Matthews *et al.,* 2006). These characteristic metabolic activities are strain-dependent and may largely vary between cultivar, or even location to some extent, as they depend on the composition of the grape berry.

Winemakers can perform MLF in various ways, and at different stages of the winemaking process. Although it represents a spoilage risk, spontaneous MLF is often performed. Indeed, most indigenous LAB strains are sensitive to sulfur dioxide, high ethanol content, low pH and low nutritional status (Bartowsky *et al.,* 2015), conditions usually fulfilled in a wine after alcoholic fermentation. For this reason, MLF can be slow or incomplete, after a long lag phase (sometimes several weeks) during which the wine must be maintained with a low content of sulfur dioxide, and thus remain vulnerable to infection by spoilage micro-organisms, as the addition of SO_2 would also compromise the development of a sufficient LAB population. Other drawbacks of spontaneous MLF are the variable production of off-flavors, acetic acid, or also the uncontrolled production of biogenic amines, allergenic compounds considered as a health hazard and subject to legal limitations (Nisiotou *et al.,* 2015). The performances of LAB are

strain-dependent and their eventual drawbacks can be avoided by the alternative to spontaneous MLF, which requires inoculation of the wine by commercial selected strains, referred to as “starter culture”.

An important advantage of conducting MLF from the inoculation of LAB, besides the choice in the strain of bacteria to be used as their performances and features can differ, is the timing of inoculation. Although sequential inoculation of LAB after the completion of alcoholic fermentation is often preferred, there is a growing interest in the practice of co-inoculation, the addition of LAB starter culture at a variable stage of alcoholic fermentation. The main advantage of co-inoculation is the reduction of the lag phase between end of alcoholic fermentation and beginning of MLF, delay during which the wine is unprotected with sulfur dioxide. When added at the beginning of alcoholic fermentation, the starter culture is thought to benefit from a progressive adaptation to the increasing ethanol concentration, and from the availability of nutrients, which results in improved performances of the bacteria (Azzolini *et al.,* 2010). The timing of inoculation should depend on the strain employed, as *O. oeni* is described as heterofermentative, meaning it can metabolize glucose into acetic acid, which would increase the volatile acidity of a wine if inoculated at the same time as the yeast, for instance. In comparison, *L. plantarum* does not present this metabolic pathway, as it is homofermentative, so it can safely be inoculated at early stages of alcoholic fermentation (Du Toit *et al.,* 2011) and perform a co-fermentation. There are evidences that the aromatic profile of wine produced by the early co-inoculation of LAB and yeast is strongly modified by such practice, leading to a significant increase in fruity aromas in comparison with sequential inoculation (Bartowsky *et al.,* 2015). This is believed to be the result of the glycosidase and esterase activity, which will tend to release more aromatic compounds present in the must under the form of aromatic precursors (Matthews *et al.,* 2006). Nevertheless, a common practice regarding co-inoculation is the addition of the MLF starter culture at a late stage of alcoholic fermentation, in order to reduce the lag phase before the beginning of MLF, with limited risk of acetic acid production (when using *O. oeni* mostly) but also with limited fruity aroma improvement by the bacteria.

During this harvest at Clos Apalta, I did not work with MLF starter cultures, as the enologist only relies on a spontaneous start of fermentation by indigenous LAB. The wines that completed alcoholic fermentation are maintained at a temperature above 30°C for a variable duration of post-fermentative maceration, before being run-off to barrels and their pomace pressed. Once placed in barrels in the “Primero año” room, and maintained there at 22°C, the wines will be carefully followed, the content of L-malic acid measured, and the organic acids composition will be monitored by paper chromatography, to assess the presence of L-lactic

acid and the consumption of L-malic acid. Once MLF has completed, the wines are racked from the gross lees, stored until the following year, and transferred before the beginning of the next harvest to the “Segundo año” room, by gravity, where they will be aged for another year before bottling.

Figure 13. Picture of the “Segundo año” barrel-ageing room.

7. 11. Production of Clos Apalta 2019

Throughout the vintage 2019 in Clos Apalta vineyards, a total of 152.21 tons have been harvested and transferred to the winery, which represents a decrease of 16.56% compared to the previous 2018 vintage. This strong variation is due to the decision of not processing the cultivar Merlot this year, due to its severe dehydration status, subsequent to the strong heat waves suffered during the second and third week of February. The consequences of this heatwave were enhanced by a relatively low rainfall during winter and spring, which did not succeed to replenish the water reserves of the subsoil. Due to the physiological characteristics of the Merlot cultivar, the vineyards were severely affected by the heatwave while grapevines were in a much more advanced physiological stage, already 3 weeks past veraison, in

opposition with Cabernet Sauvignon, Petit Verdot and Carménère, much later varieties. The early variety Merlot suffered a severe phenolic maturation blockage, inducing difficulties to obtain ripe tannins, a serious decrease in colour, as well as a rather low accumulation in aromatic precursors. It appears that the Merlot vines, following a systematic basal leaf removal, suffered from an excessive exposure of the cluster area to intense irradiation (indeed, the canopy wasn't particularly damaged by the heat), leading to the berries' overheating and the thermal degradation of skin's proteins, and a phenomenon of berry shriveling, likely to be triggered by a backflow of water from the berry back into the plant (Gutiérrez-Gamboa *et al.,* 2019). This severe dehydration could not be recovered along the rest of the growing season.

Figure 14. Picture of Merlot grapevines taken 3 weeks after the heatwave, illustrating the degree of dehydration of the berries.

Plots showing low vigor were the most affected by these extreme conditions and the resulting damage to the plants was substantial. All vineyards of Clos Apalta are planted on the slope of the valley, and even though they are mostly irrigated, the vigor of the vineyard is maintained low to obtain ripe concentrated fruits. As showed on Figure 14, Merlot clusters were extremely dehydrated, sometimes entirely composed of dried berries, and the decision was taken not to process Merlot for the production of Clos Apalta this year. However, the plots have partially

been harvested and the rather low quality grapes (in total, only 23.25 tons compared to 48.96 in 2018) have been sold to Lapostolle winery, to balance with the unfortunate economical loss induced by the heatwave.

Nevertheless, the winery sells grapes to Lapostolle winery on a regular basis, since the production of the vineyards exceeds the capacity of the winery. In a difficult year like 2019 with complicated climatic conditions affecting the production of the vineyards, the direction simply decided not to sell as much grapes as an average year, to balance with the partial loss of crop. Consequently, the production of Cabernet Sauvignon has entirely, and not partially, been processed by the winery leading to an increase of 66.95%. Indeed, 20.82 tons of Cabernet Sauvignon were sold last year, but the overall yield of the vineyards saw an increase of 19.78% between 2018 and 2019. Besides, Clos Apalta also buys Carménère grapes every year from a neighboring producer (named Jorge Azua) which produces exceptional qualitative crop. This year, the amount of grapes bought by the winery was increased by 204.90%. Therefore, the total amount of grapes received and processed at Clos Apalta was increased by 24.28% to counterbalance the loss of the entire production of Merlot, although parts of it was sold but however, the overall vineyards production decreased by 12.82%.

Table 10. Weight of grapes (in tons) received in 2018 and 2019, with variation between vintages.

	Produced			**Received**		
	2018	**2019**	**Variation**	**2018**	**2019**	**Variation**
Merlot	48.96	23.25	-53%	48.96	0	-100%
Cabernet Sauvignon	66.68	79.87	19.78%	47.84	79.87	66.95%
Carménère - Clos Apalta	79.7	66.82	-16.16%	79.7	66.82	-16.16%
Carménère - Azua				41.25	125.77	204.90%
Petit Verdot	5.92	5.52	-6.76%	5.92	5.52	-6.76%
Total:	201.26	175.46	**-12.82%**	223.67	277.98	**24.28%**
Total Originated from Clos Apalta vineyards:				182.42	152.21	**-16.56%**

Nevertheless, not all varieties suffered the heatwave so severely, and except some plot without irrigation, Cabernet Sauvignon was picked with an overall satisfactory maturity, with harvest starting on the 20th of March and ending on the 11th of April. Some non-irrigated plot, mostly on steep slopes with poor soil and low vigor, suffered slight dehydration and anthocyanins thermal degradation resulting in berries of low colour and high sugar content.

Besides these casualties, most plots planted with Cabernet Sauvignon produced qualitative crop with relatively high sugar content, but with total acidities comprised between 4 and 5 g/L of tartaric acid equivalent, and a satisfying phenolic maturity. Carménère also performed well regarding phenolic maturity and ripeness balance, although the yield has been significantly reduced since the previous vintage, by 16.16%. Harvested between the 15th of April and the 11th of May, its characteristics varied a lot along the course of almost an entire month of harvest. Most of the Carménère picked was rather low in acidity (3 g/L of tartaric acid equivalent) and high in sugar, up to 16% of potential alcohol, but this rather late harvest is a necessity for the profile of wines expected at the Clos Apalta winery, with low herbaceous character and high maturity (Sala *et al.,* 2004).

Example of Fermentation: Cabernet Sauvignon

The following description concerns a batch issued from the plot 26, with Cabernet Sauvignon planted in 2006 at the density of 6666 plant/ha, on the rootstock 101-14 Mgt known for a moderate tolerance to drought (Carbonneau, 1985).

Table 11. Reception estimations (top) and must analysis (bottom) for Cabernet Sauvignon cv. from plot 26.

Code: CS 19/053 - Plot 26	
Weight Total (kg)	8411
Weight Discarded (kg)	992
Weight Tank (kg)	7419
Volume Tank (L)	5415
Volume Saignée (L)	810
Volume Final (L)	**4605**

Potential Alcohol (%vol.)	14.01
Temperature (°C)	20.3
Density	1102
TA (g/L tartaric acid)	4.35
pH	3.55
YAN (mg/L)	59.71

With a potential alcohol of 14% vol., a rather cool and slow fermentation was desired, to enhance the aromatic character of the wine without risking a complicated kinetics at the end

of fermentation. The acidity of 4.35 g/L conferred a fresh mouthfeel which did not require any acid correction. The YAN content was rather low and required a first addition of organic nutrient Fermaid-K (20 g/hL) and DAP (20 g/hL) at the density of 1080, and a second addition of DAP only (15 g/hL) at the density 1040. Délestages were performed twice, at the density 1085 and 1050 to enhance phenolic extraction, and remontage with aeration were performed 4 times per day, throughout alcoholic fermentation, along with punch-downs, 6 times daily.

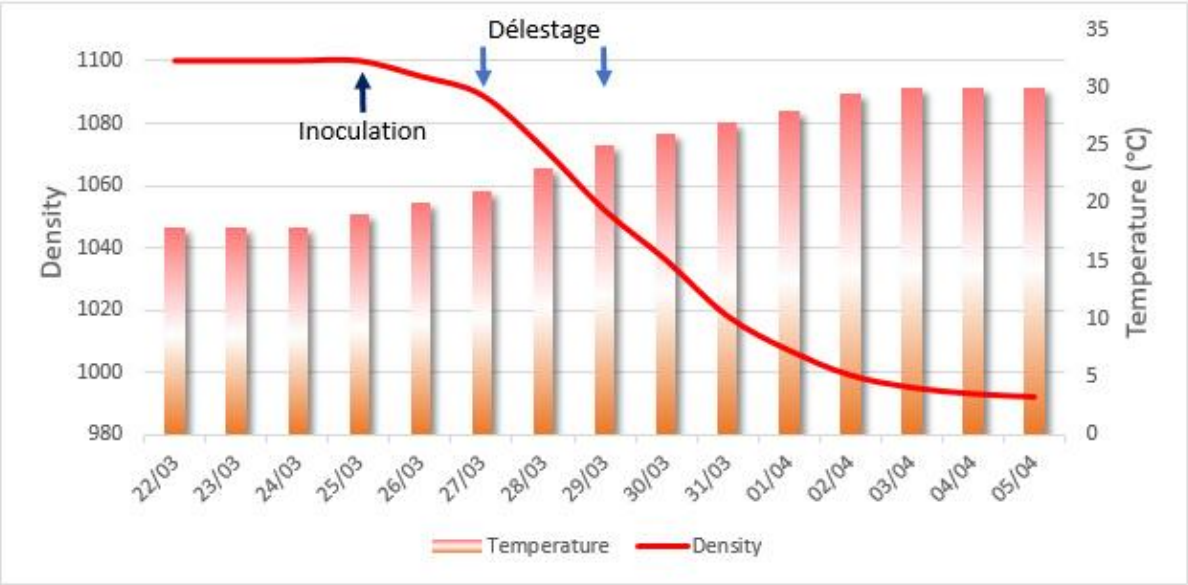

Figure 15. Fermentation curve of Cabernet Sauvignon from plot 26 in vat C-09, inoculated with Zymaflore F15.

The yeast Zymaflore F15 did not show any sign of difficulty along the alcoholic fermentation, and a slight reduction was only detectable before the second nutrition, but the addition of DAP along with a cycle of remontage with aeration helped fixing this situation. Tannin extraction was evaluated at the end of fermentation and considered satisfying, so the temperature was later set at 31℃ to promote polymerization and produce a rounder mouthfeel. At this point, remontage were not performed but a simple circulation of the wine between the 2 front valves was operated, only to homogenize the temperature and replace the settling yeast in suspension to complete metabolizing any residual sugar.

Table 12. Chemical analysis of CS 19/053 wine after fermentation completion.

Alcohol (%vol.)	13.9
Residual Sugar (g/L)	3.12
Titrable Acidity (g/L)	4.11
pH	3.62
Volatile Acidity (g/L)	0.35
Free SO2 (mg/L)	6.29
Total SO2 (mg/L)	29.31

The Cabernet Sauvignon wine was fruit-oriented, with aromas of blackberry and cherry. Fresh with a long finish, this wine will hopefully evolve into a round structured wine, able to bring fresh fruits to the final blend. After the chemical analysis presented in Table 12, the Volatile Acidity (VA) and SO_2 level were assessed, and the wine was racked, transferred to brand new oak barrels (from Radoux, Baron and Seguin Moreaux) where it will undergo malolactic fermentation.

Example of Fermentation: Carménère

This example concerns the vinification of a batch of Carménère, originated from the plot 216 planted on its own roots in 2005, at the density of 6666 plants/ha. The vineyard is situated on a south-east facing slope, benefiting from a limited irradiation which enables a rather long maturation phase until satisfying phenolic and aromatic maturity. The grapes from this batch were placed in 22 barrels, filled with 200 kg each, to perform barrel fermentation.

Table 13. Must analysis for Carménère cv. from plot 216.

Code: CA 19/252 - Plot 216	
Potential Alcohol (%vol.)	16.21
Temperature (°C)	19.2
Density	1118
TA (g/L tartaric acid)	3.64
pH	3.88
YAN (mg/L)	68.05

Carménère is a variety originated from the Bordeaux area in France, which tends to present a high content of methoxypyrazines, responsible for a strong herbaceous aroma (Botezatu *et al,* 2016). In the Bordeaux area, Carménère is difficult to ripen and often too green. In the Colchagua valley, the climate allows an advanced maturation, and with proper vineyard management, it is possible to obtain grapes with low levels of methoxypyrazines, but for this, it is recommended to harvest it at high degree of maturity. For this reason, sugar content and potential alcohol tends to be quite high, and acidity rather low, although cool nights help maintain it. After analyze, the decision was taken to correct the acidity of the must, slightly buffering with such high pH, and lower the sugar content by adding water. A saignée of 20% of the expected volume of wine (73% of 200 kg of grapes, equivalent to 146L of wine) was drawn, corresponding to 29L, and 5.5L of water added with 50g of tartaric acid was poured into the barrel and mixed to the must. The acid addition corresponded to an increase of 0.42 g/L of tartaric acid, and the water addition decreased the density by 14 units. The target of the enologist regarding acidity in Carménère is 4 g/L, and the water addition was aiming at lowering the potential alcohol below 15.5%vol.

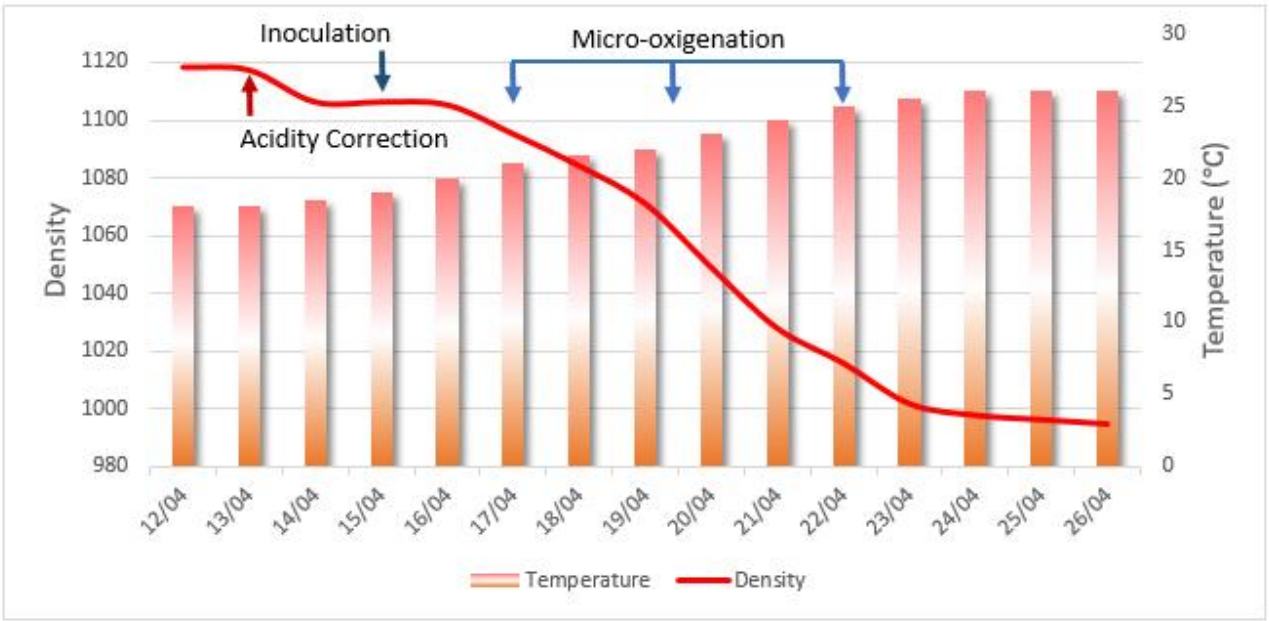

Figure 16. Fermentation curve of Carménère from plot 216 in barrels CA 19/252, inoculated with IOC R9008.

After the adjustments of acidity and sugar of the must, and a cold maceration maintain for 4 days, with daily punch-down and rotation of the barrels to hydrate the cap, the barrels were added with the yeast IOC R9008. Known for its performances in difficult conditions such as high sugar content, this very osmotolerant yeast is well suited for warm climate's fruits allowed

to fully ripen. Nutritions were necessary due to the low YAN content of the must, and it was separated in 2 additions. The organic nutrition was performed at the density of 1086 with 25 g/hL of Fermaid-K, along with 25 g/hL of DAP. The second nutrition consisted of 15 g/hL of DAP at the density 1040. Micro-oxygenation was also necessary to fulfil the yeast's oxygen requirements. A first spurge of 60sec at 1 bar of pressure was performed at the density 1086, after the first nutrition, followed by a second spurge of 30sec at the density 1050, and a third spurge of 10sec at the density 1020. Daily punch-downs and rotation were maintained until the end of alcoholic fermentation, and the barrels were finally drained from the free run wine, and the pomace was sent to the basket press. Free run wine and Press wines were transferred for malolactic fermentation, separately, into the barrels used for alcoholic fermentation (Saury Premium M Carménère) and 2 new barrels from Orion (Allier) were filled with part of the Free run wine, to intensify the oak aroma contribution. The Carménère wine was a full-bodied, round and rich wine with supple tannins even at such young age, a deep ruby colour and aromas of ripe blackberries, black currant and a hint of smoke.

Table 14. Chemical analysis of CA 19/252 wine after fermentation completion.

Alcohol (%vol.)	15.29
Residual Sugar (g/L)	3.37
Titrable Acidity (g/L)	4.08
pH	3.52
Volatile Acidity (g/L)	0.43
Free SO2 (mg/L)	4.04
Total SO2 (mg/L)	21.36

8. Issues Encountered and Control Methods

8. 1. Stuck Fermentations

This vintage in the Colchagua valley has presented one particular challenge for many enologists of the area, namely a rate of stuck fermentation much higher than previous years. The reason is still poorly understood, although there are evidences for the influence of the ratio Fructose/Glucose in the berries (Malherbe *et al.,* 2007), leading to complicated fermenting conditions during the later stage of alcoholic fermentation. Contact with suppliers of enological products confirmed that the demand for stuck fermentation aides has been significant this vintage, to the extent of completely running out of stock for some additives,

such as Bi-Active® from Laffort, for instance. Through adsorption of fermentation inhibitors such as medium-chain fatty acids, the yeast cell walls that compose this additive act like a "detoxifier" of the fermenting wine. It is also composed of cellulose, which helps the fermenting yeast remain in suspension. This product was completely sold out by one national leading distributor by the middle of May, sign of an exceptional rate of stuck fermentation.

Various methods can be implemented to counteract such complicated end of fermentation. The most systematic technique to help complete alcoholic fermentation relies on the "Pied de Cuve" method described in Chapter "6.4. White Wine Fermentation" in a different context. It can be used in 2 different ways according to grapes reception, or more precisely, according to the availability of must or not. If the stuck fermentation is detected early enough, while grapes are still being harvested and received, it is possible to prepare a Pied de Cuve from freshly harvested grapes. In a separate tank, a volume of must (corresponding to 5-10% of the volume of the stuck tank) can be added with a high content of Nitrogen from both organic and inorganic nutrition additives, and inoculated with a large population (30 – 40 g/hL) of a strongly fermenting and ethanol tolerant yeast, such as *Saccharomyces bayanus* strains. The fructophilic character of this strains makes it a good candidate for completing alcoholic fermentation, since most of the glucose has already been consumed during earlier stages of the fermentation (Malherbe *et al.,* 2007). In such conditions, the population of yeast will rapidly ferment the sugar available in the must, and once the density of this small volume of must is near the density of the stuck tank, the Pied de Cuve can be added to the tank. Conditions of similar temperatures and densities help limiting the osmotic or thermic choc the yeast would otherwise suffer from. Because of dilution effect, the kinetics of fermentation will inevitably drop significantly, but the population of yeast will be able to slowly carry on with alcoholic fermentation (Henschke, 1997).

If stuck fermentation occurs later in the season, when harvest is complete, only wines are available to prepare a Pied de Cuve. These circumstances are those occurring in sparkling wine production, following the "Méthode Champenoise", or "Méthode Traditionnelle" at the stage of "Tirage", where a Pied de Cuve preparation is added to trigger a secondary fermentation in bottle. It consists in preparing a Pied de Cuve, similar to the method explained above, but using wine, and in our case wine in stuck fermentation. Developing the yeast population will first require to dilute 5-10% of the wine until reaching 8% of alcohol strength, and if necessary, to enrich it with sugar (at least 20 g/L) in order to induce alcoholic fermentation before adding the Pied de Cuve to the stuck wine. It is indeed important to obtain good fermentation kinetics before adding the Pied de Cuve to the wine, where the difficult environment would otherwise compromise the success of this method. The wine can be added

with commercial preparations such as detoxifiers (Bi-Active® from Laffort) to ease the yeast's adaptation, and while rehydrating the yeast, the addition of commercial nutrition aides is recommended. Composed of sterols, vitamins, long chain fatty acids, preparations such as Nutrient Vit Nature® from Lallemand or SuperStart Spark® from Laffort can help obtain a yeast population with healthy membranes able to adapt to high ethanol content and hostile environments. Once this preparation shows an active fermentation, with good kinetics, and a minimum density of around 1000 (lower density would see a decrease in activity of the yeast), the Pied de Cuve can be added to the stuck tank, and homogenized by a pump-over of the entire volume of the tank.

Other methods exist to counteract a stuck fermentation. For instance, during this vintage at Clos Apalta, 2 vats have been chosen for an experiment aiming at completing stuck fermentations without re-inoculation. Both from the Carménère variety, from nearby plots, one vat (C-10) was stuck at the density of 998, with 25 g/L of residual sugar, and the other vat (C-02) was more recently harvested, and at the peak of its fermentation. The stuck vat was drained from its wine, stored in a temporary tank, and the fermenting wine from C-02 was transferred onto the pomace of C-10. Then, the stuck wine from C-10 was transferred onto the pomace of C-02. This corresponds to a simple exchange of wines from both vats, onto the pomace of each other, and aims at adding active yeast to the stuck wine, from the lees remaining in the vat. It required some time (5 days) but fermentation restarted and was completed in the end. The main drawback of such method is the interruption of phenolic and aromatic extraction by the younger of the 2 wines, which is transferred during its maceration onto a pomace of much more advanced maceration stage. Subsequently, the previously stuck wine will be allowed a much more intense maceration, on 2 different pomaces.

The delay during which there is no active fermentation represents a significant risk of infection by spoilage micro-organisms. The activity of Acetic Acid Bacteria can pause a problem, and the volatile acidity of such wines running into a stuck fermentation tends to be quite high. C-02 finished its alcoholic fermentation with a volatile acidity of 0.59 g/L, almost 30% higher than other vats' average. Another risk of infection in such delayed fermentation conditions concerns the development of the spoilage yeast *Brettanomyces bruxellensis.* Both risks of spoilage are described below.

8. 2. Acetic Acid Bacteria

In parallel with the issue of stuck fermentations on red wines, the impact of Acetic Acid Bacteria was previously illustrated by an experiment conducted on white wines. While shortly working at Lapostolle winery, I participated to one microvinification experiment of spontaneous fermentation that started prior to my arrival, on Sémillon grapes from a recently planted vineyard. The aim of this experiment was to assess the potential of such fermentation in the context of the new vineyard, to promote aromatic complexity through a short skin maceration which could also affect the colour and mouthfeel of the wine. This experiment was conducted in parallel with a wine from the same Sémillon grapes, pressed and inoculated with a neutral yeast from the *Saccharomyces bayanus* genus, the IOC 18-2007. I unfortunately did not work there long enough to observe the evolution of this wine, or to assess the final results and compare the eventual variation between the 2 wines. Nevertheless, the final chemical analysis pointed out the fact that the wine produced by spontaneous fermentation presented a volatile acidity of 0.82 g/L, above the detection threshold of 0.7 g/L according to Amerine & Roessler (1980). It is likely to be a consequence of the relatively high production of acetic acid and ethyl-acetate by wild yeast, as well as the presence and activity of acetic acid bacteria.

Table 15. Chemical analysis of the 2 Sémillon microvinification experiments.

	Pied de Cuve	IOC 18-2007
Alcohol (%vol.)	13.4	13.7
Residual Sugar (g/L)	2.4	1.6
Titrable Acidity (g/L)	4.62	4.81
pH	3.73	3.68
Volatile Acidity (g/L)	0.82	0.51

Acetic acid bacteria (denoted AAB), Gram-negative bacteria belonging to the *Acetobacteraceae* family, are widely used for the production of vinegars. However, their uncontrolled activity can have serious consequences on wine, as they are considered a spoilage microorganism. They are well adapted to environment rich in ethanol or sugar, and their resistance to sulfur dioxide is moderate, although a sufficient amount can substantially limit their activity. The main strains responsible for wine spoilage are *Glucanobacter oxydans*,

well adapted to sugar-rich environment but usually disappear during alcoholic fermentation, and *Acetobacter aceti,* very tolerant to ethanol so remain active after completion of fermentation (Du Toit & Lambrechts*,* 2002).

These bacteria are aerobic and their growth and proliferation requires oxygen, so an attentive care should be taken by winemakers regarding exposure of the wine to air. In aerobic conditions, they can oxidize ethanol into acetaldehyde and convert it to acetic acid. Once spoiled by AAB, wine shows an increase of a characteristic volatile acidity, similar to that of vinegar, a nail polish remover aroma as well as a sourness and reduction in body and fruity character (Bartowsky & Henschke, 2008).

During fermentation, the anaerobic conditions as well as the presence of free sulfur dioxide are efficiently limiting the development and activity of AAB, but their requirement for oxygen can be fulfilled during ageing and storage. The volatile acidity of wine will also tend to increase during wine ageing in oak, resulting from extraction of acetic acid from the wood, and from oxidation reaction of ethanol into acetic acid due to micro-oxygenation. The reaction of ethanol and acetic acid produces ethyl-acetate (Nishimura *et al.,* 1983), which also contribute to the sensory perception of volatile acidity, so a particular attention regarding the evolution of these compounds should be taken during wine ageing in wood.

With the current trend of diminution of sulfur dioxide in wine, spoilage by AAB could become a common issue. It is important to mention that the concentration of acetic acid found in wine is also subject to legal limitation, currently 1.2 g/L (OIV, 2017). Due to careful sulfur management, CO_2 protection during cold maceration and repeated punch downs or pumping overs on a daily basis, volatile acidity is usually found between 0.3 and 0.5 mg/L in wines after alcoholic fermentation at Clos Apalta. Since malolactic fermentation is mostly conducted in brand new oak, as well as 2 years of oak ageing before bottling, the volatile acidity can slowly increase to values near the detection threshold of 0.7 mg/L (Amerine & Roessler 1980). in some wines, although it usually remains below 0.6 mg/L. The final blending, besides producing a complex wine with complemented aromas and a balanced taste, will also aim at obtaining a final volatile acidity below the detection threshold in order to remain mostly undetectable by the majority of consumers.

8. 3. Brettanomyces

Maintaining barrels completely full is a priority during barrel ageing, as it limits oxidation of the wine (with a slow micro-oxidation preferred) and it also reduces the development of acetic acid bacteria, lowering the kinetics of volatile acidity apparition. Consequently, barrel refilling is a frequent duty in the winery, as is control of SO_2 and its correction when the levels are judged low (during ageing, the Free SO_2 must be maintained above 25 mg/L). This barrel's inspection and refilling is an opportunity to tend to the evolution of the wines during ageing, and to pay attention to any suspicious aromatic deviation. This last duty was decisive on the 12th of May 2019, when a barrel (18/259b) was found to present an aroma reminiscent of barnyard, possible sign of spoilage by the yeast *Brettanomyces bruxellensis.* A sample analyzed under the microscope revealed the presence of a large population of the undesired yeast, as presented in the Figure 17.

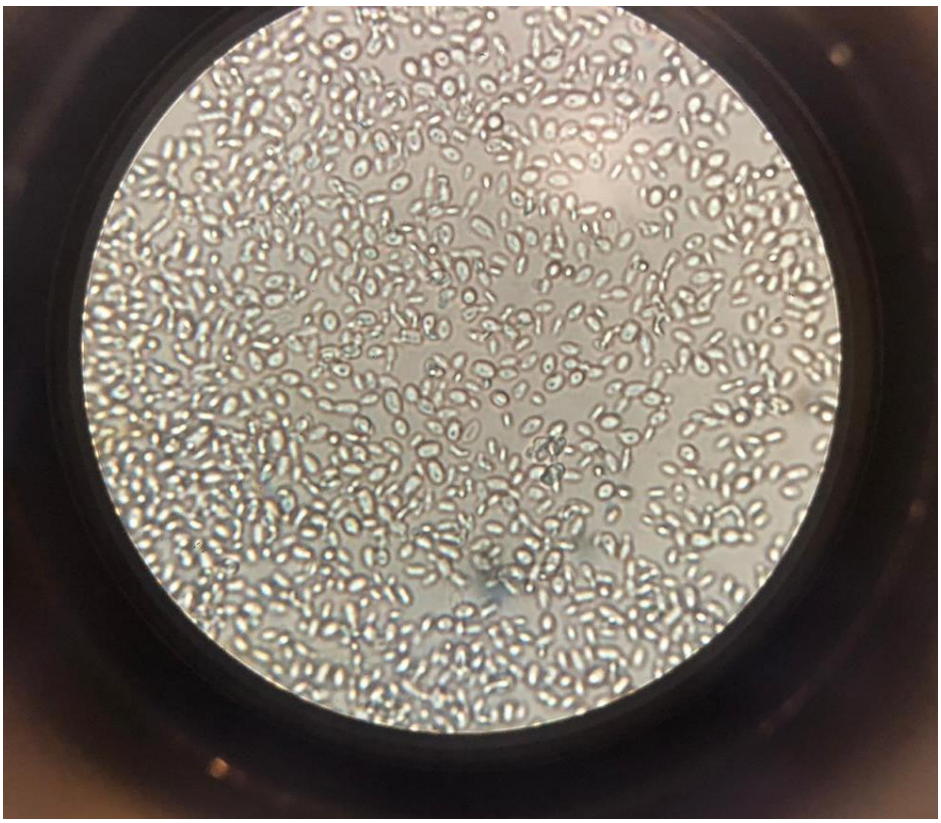

Figure 17. Picture of the sample of wine 18/259b observed by optical microscope, with a magnification of 1000X.

Amongst all spoilage microorganisms encountered in wine, yeast from the *Brettanomyces* genus (or its teleomorph *Dekkera*) is probably the most predominant source of wine spoilage,

especially *Brettanomyces bruxellensis,* particularly active in red wines. Due to its exceptional adaptation to ethanol, sulfur dioxide, low oxygen, low nutrition and low pH, *B. bruxellensis* is representing a considerable risk of wine spoilage, especially red wines aged in oak casks, potentially causing serious economic loss to the producer (Loureiro & Malfeito-Ferreira, 2003).

The presence of *Brettanomyces* is minimal in the vineyard, but poor sanitary conditions of winery equipment provides the support where it can survive until the next vintage, mostly as a teleomorph organism. Barrels are its preferable niche, where the microporous structure of the wood offer a safe environment for survival and complicates the efficiency of cleaning. During the early stages of winemaking, grape musts are dominated by rapidly fermenting yeasts with high speed of multiplication, such as *Saccharomyces cerevisiae,* which prevails until depletion of its main substrate, glucose. After the completion of alcoholic fermentation, the conditions of high ethanol content and low available nutrition favors the slow development of *Brettanomyces* (Tubia *et al.,* 2018).

Beside producing variable amounts of acetic acid, *Brettanomyces* is mostly recognizable for its ability to use hydroxycinnamic acids present in must and wine, specifically *p*-coumaric and ferulic acids, and convert them into 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG) respectively. The conversion of these phenolic acids into their respective volatile phenols strongly affects the wine's aroma, as it produces off-flavors usually described as "horse sweat", "barnyard", "smoky" and "medicinal", and damages the fruity character of the wine (Suárez *et al.,* 2007). It is important to mention that the use of some pre-fermentative pectolytic enzymes displaying cinnamoyl esterase activity can increase the levels of hydroxycinnamic acids in wine, and then intensify the impact of *Brettanomyces* activity (Tubia *et al.,* 2018).

Malolactic fermentation also tends to increase the risk of apparition of these volatile phenols. Indeed, some strains of Lactic Acid Bacteria (LAB) can feature a cinnamoyl esterase activity, releasing cinnamic acids previously esterified with tartaric acid, and even a decarboxylase activity to produce vinyl-phenols that *Brettanomyces* can ultimately reduce into the ethyl-phenols mentioned above. Some strains of LAB have even been found to be able to reduce vinyl-phenols into ethyl-phenols, and thus produce the off-flavour usually attributed to *Brettanomyces* (Silva *et al.,* 2011). Therefore, malolactic fermentation can increase the concentration of substrate for such spoilage micro-organism, and intensify the risk of affecting the aroma of a wine, in case of infection. It is however possible to perform malolactic fermentation with commercial strains of LAB, selected for their lack of such enzymatic activity. It is also possible to remove *Brettanomyces* with reasonable efficiency with the use of oenological additives, such as Chitosan (Bağder-Elmacı *et al.,* 2015).

8. 4. Chitosan

Chitosan is a bioactive polymer obtained through deacetylation of Chitin, the second most abundant polysaccharide in nature after cellulose. Therefore, this macromolecule being derived from renewable and available sources, biodegradable and biocompatible, Chitosan is widely investigated for biomedical application, and more recently for enological application (Colangelo *et al.,* 2018). The use of Chitosan, extracted from the fungi *Aspergillus Niger*, has been authorized in winemaking by the EU since 2011.

There has been an increasing interest in potential applications of Chitosan in winemaking, particularly due to its wide spectrum of activity. Until recently, it was mostly used to improve wine safety by reducing toxic compounds concentration. Effective for heavy metal removal, especially lead (Pb) and cadmium (Cd) but also iron (Fe) involved in ferric casse, it also allows a significant reduction of Ochratoxin A (Bornet & Tesseidre, 2007) and the prevention of protein haze in white wine (Chinnici *et al.,* 2014). There is evidences that Chitosan can improve the sensory profile of wines affected by *Brettanomyces,* by reducing the headspace concentration of volatile phenols. While there was no evidence of reduction of 4-EP and 4-EG in the wine, the headspace concentration was decreased by 26% (Filipe-Ribeiro *et al.,* 2018) meaning that Chitosan represents a suitable fining agent regarding adsorption of volatile phenols.

Regarding the control of wine spoilage microorganisms, Chitosan shows promising results as it completely inhibits the development of spoilage yeasts such as *Brettanomyces bruxellensis* from concentrations as low as 0.2 g/L, while *Saccharomyces cerevisiae* exhibits a relatively high resistance up to 2 g/L (Bağder-Elmacı *et al.,* 2015). Nevertheless, lactic acid bacteria responsible of malolactic fermentation also seem to be inhibited by Chitosan, so its use should be correlated to the style of wine to be produced. Furthermore, the treatment with Chitosan was found to be effective in the prevention of another microbiological spoilage, volatile acidity. Not only inhibiting the growth of AAB, it has proved to be efficient in reducing their metabolic activity and even their viability (Valera *et al.,* 2017).

In this occurrence, a preparation of Chitosan commercialized by Lallemand under the name "No Brett Inside™", presented in Figure 18, was used as soon as the spoilage was detected in the barrel 18/259b. This preparation of Chitosan of fungal origin (*Aspergillus Niger*) inactivates *Brettanomyces bruxellensis* which settles over some time (10 days are recommended by the manufacturer) and can be removed by racking the wine from its lees.

"No Brett Inside™" was thoroughly mixed in 5 times its volume of water, at the dosage of 5 g/hL (hence 11.25 grams for the barrel), and added to the wine while stirring to ensure its distribution through the entire volume. As recommended, the wine was racked from its lees after 10 days and transferred to a freshly cleaned and sterilized barrel. However, the Chitosan treatment only allows to remove the spoilage yeast, but it has no impact on the levels of volatile phenols already produced. Unfortunately, the spoilage was detected at a rather advanced stage, and even the use of Chitosan could not fix the organoleptic damage caused by *Brettanomyces bruxellensis.* It is important to mention that enological products such as "No Brett Inside™" are very expensive, so a proper use of sulfur, deep cleaning of barrels and attentive care during wine ageing would limit the economic loss induced by such spoilage, especially substantial in case of irreversible damage to the wine.

Figure 18. Picture of the commercialized Chitosan preparation "No Brett Inside™" 100g, by Lallemand.

8. 5. Sulfur Dioxide

The use of sulfur dioxide (SO_2) for its antioxidant and antiseptic properties, enhanced at wine's usually low pH (Ribéreau-Gayon *et al.,* 2006e), has been a well-known solution against spoilage by undesired yeast and bacteria since the roman era, and almost a systematic solution since the 18th century. We are facing nowadays a new challenge, as the toxicity of

sulfur dioxide has become of major interest, and regulations by OIV (International Organization of Vine and Wine) tend to decrease its legal limits. To comply with updated regulations regarding the use of sulfur dioxide, a cautious approach is necessary to ensure sufficient stability of the wine regarding microbial spoilage and oxidation, without legal compliance issues.

For convenience of use, sulfur dioxide is often preferred under its aqueous form, diluted with water to a specific concentration. Like many other wineries, Clos Apalta works with a solution of SO_2 at the concentration of 5%, limiting the hazard it represents in case of contact with the skin or eyes of the workers. The preparation of sulfur solution consists in filling a tank with water (water without Chlorine is preferred, so tap water isn't usually used for this purpose). This tank must be able to withstand pressure, and be equipped with a safety pressure-releasing valve. A pressurized SO_2 cylinder tank is then connected to a spurge, placed in the center of the tank. Once opened, the inlet spurges gaseous sulfur dioxide through the water inside of the tank, slowly increasing the SO_2 concentration of the solution. The concentration of the solution can be monitored by a simple density measurement, where the target density must be known. Here, the target is to obtain a solution of 5% SO_2 so the density of the final solution must be 1028 at 20°C. Other methods of sulfur dioxide's use include flushing wine/must with gaseous SO_2 (less common because of difficulties to monitor the quantity added to the wine) or also direct addition of potassium metabisulfite salts, either in tablets or powder.

As part of my work at Clos Apalta, I elaborated the easy calculation digital tool presented below, in Figure 19. Taking into account the volume of wine or must to protect, as well as the concentration of SO_2 solution employed at the winery, it also requires description of basic chemical parameters measured in the Laboratory, such as Alcohol content (% vol.) or Potential alcohol in case of must, pH and Temperature. With these parameters adjusted, the calculation tool can determine the percentage of Active (or Molecular) SO_2 in such conditions, using the formula of Sudraud & Chauvet (1985):

$$\%\text{Molecular SO2} = 100 / 10^{(pH - 1.81)} + 1$$

From this information, the tool offers the choice of initial addition of SO_2 in g/hL according to the enologist, and it expresses the Free SO_2, Total SO_2 and Active SO_2 at such concentration, along with the volume of SO_2 solution required for such addition, taking into account its dilution factor.

The second section of the tool, entitled "Correction SO_2" concerns a finished wine, which requires protection for a safe ageing. The enologist is free to decide which concentration of Active SO_2 he/she considers safe during wine ageing, as the tools calculates the concentration of Active SO_2 based on the values entered as "Initial" and "Target" Free SO_2, and expresses the volume of SO_2 solution required to reach the Target of Free SO_2. The concentration of Free SO_2 considered efficient against the development of *Brettanomyces bruxellensis* is variable according to the study but it usually ranges between 25 mg/L (Du Toit *et al.,* 2005) and 40 mg/L (Barata *et al.,* 2008), according mainly to the pH of the wine. A lower pH will induce a higher dissociation of Molecular SO_2 and result in a higher antiseptic action, thus limiting the initial amount necessary to inactivate the spoilage yeast. The efficiency can be variable, as it depends on the composition of the wine matrix. It can be calculated along laboratory measurement, by adding a given quantity of SO_2, and after a delay of 24h or more, the ratio Free SO_2 / Total SO_2 is the efficiency considered for this particular wine. The default value considered in most studies is 2/3, or 66% (Ribéreau-Gayon *et al.,* 2006e).

INITIAL ADDITION SO2

Volume (L)	Alcohol (%vol.)	pH	T(°C)
10,000	14	3.66	22.0

Solution SO2 Concentration
5%

Addition (g/hL)	2
Free SO2 (mg/L)	13.2
Total SO2 (mg/L)	20
Active SO2 (mg/L)	0.35
Volume SO2 (L)	4.0

CORRECTION SO2

		SO2 Active	2.6%
Initial Free SO2 (mg/L)	10	0.3	
Target Free SO2 (mg/L)	25	0.7	
% Efficiency	66%		
Volume SO2 to add (L)	4.55		

Figure 19. Calculation tool for Initial SO2 addition and Correction of SO2 based on characteristics of the must/wine.

Solid sulfur is also widely used to sanitize barrels and wooden vats under the form of sticks or discs. Suspended into the wooden vessel, previously rinsed and dried, the disc/stick of sulfur is burnt, and the smoke is allowed to occupy the closed space. The quantity recommended is 2 g/hL, and most wineries use 5 grams for one barrel of 225L. Here at Clos Apalta, 10 grams are used to sanitize barrels, for microbiological safety purpose, since mostly new oak is used for the ageing of red wines. The barrels are later stored for extended periods of time, transferred to the other winery of the company (Bodega Lapostolle) in which case, the enologist prefers to ensure safety towards spoilage micro-organisms. However, this safety measure of doubling the amount of sulfur burnt into the barrels represents a substantial cost every year. However, when the barrels are to be reused in a short time (maximum 1 week) for instance at the Lapostolle winery, only 5 grams of solid sulfur are being used, and either way, right before usage, 2.5 g of sulfur are burnt again as a preventive measure, unless the Free SO_2 content of the wine to be transferred is satisfactory.

An important parameter to take into account when burning sulfur discs or sticks into a wooden vat is the necessity to perform this operation once the vat is dry. Indeed, the main difference between barrels and wooden vats is the presence of stainless steel equipment inside of the vessel. If sulfur is burnt inside of a wet wooden vat, gaseous SO_2 will react with H_2O to form sulfuric acid (H_2SO_4), extremely corrosive, and this reaction would strongly deteriorate the stainless steel equipment such as valves, doors and cooling serpentine, thus resulting in large economical loss.

9. Conclusion

The Master's thesis represents an opportunity to consolidate the theory background provided by the Vinifera Master of Science in Viticulture and Enology. As such, this professional experience strongly requires to apply a thorough analyze and critical observations in order to understand, describe and sometimes improve the procedures involved in grape processing and wine production alike.

Clos Apalta offered me the opportunity to learn about the caution, precision and intensity involved in the production of premium red wines aiming for long ageing potential, with the extensive use of French oak, vats and barrels, throughout wine's production. Cautious decision of harvest, precision in berries' selection, intensive cap management practices alongside sustained oxygenation are all involved in the optimization of phenolic content and stability, aromatic extraction and overall organoleptic intensity and complexity. Although such

extensive use of oak is a matter of debate, as its impact can often overpower the fruit's contribution to the wine's aroma, flavor and mouthfeel, the resulting high content of hydrolysable tannins extracted from oak can, however, benefit the wine's ageing potential by their antioxidant action. This intensely oaked wine profile might not be accessible to all consumers, but it is undeniable that many iconic wines from around the world share these features.

The management aspect of this professional experience was a valuable opportunity to improve my skills as a future wine industry executive. Indeed, the skills required for leading a team are a necessary feature, while ensuring that the wines are well tended to and that no duty is being overlooked is often a challenge. Furthermore, the position of Assistant Winemaker that I was lucky to be offered allowed me to take part in the decisions regarding enological aspects of the production and the careful monitoring of the evolution of the wines during fermentation and ageing.

The overall learning outcome of this professional internship coupled with the development of this Master's thesis was exceptionally valuable, as the analytic aspect required by the production of such document undoubtedly optimized this experience. I can only feel grateful for the opportunity I've been given to work as Assistant Winemaker in such an emblematic winery, and for the evolution in my understanding of wine production throughout this experience.

Figure 20. Picture of Clos Apalta winery.

References

Aguilera, F., Peinado, R. A., Millan, C., Ortega, J. M., & Mauricio, J. C. (2006). Relationship between ethanol tolerance, H+-ATPase activity and the lipid composition of the plasma membrane in different wine yeast strains. *International Journal of Food Microbiology*, *110*(1), 34-42.

Aleixandre-Tudo, J. L., & du Toit, W. (2018). Cold maceration application in red wine production and its effects on phenolic compounds: A review. *LWT - Food Science and Technology, 95*, 200-208.

Allen, M., Leske, P., & Baldwin, G. (1997). Advances in juice clarification and yeast inoculation: World Congress Centre, Melbourne, 15 August 1996: Australian Society of Viticulture and Oenology.

Amerine, M. A., & Roessler, E. B. (1980). *Wines: Their Sensory Evaluation*. New York: W. H. Freeman and Company.

Azzolini, M., Tosi, E., Vagnoli, P., Krieger, S., & Zapparoli, G. (2010). Evaluation of technological effects of yeast-bacterial co-inoculation in red table wine production. *Italian Journal of Food Science*, *3*(22), 257-263.

Bağder-Elmacı, S., Gulgor, G., Tokatlı, M., Erten, H., Isci, A., & Ozçelik, F. (2015). Effectiveness of chitosan against wine-related microorganisms. *Antonie Van Leeuwenhoek, 107*(3), 675-686.

Barata, A., Caldeira, J., Botelheiro, R., Pagliara, D., Malfeito-Ferreira, M., & Loureiro, V. (2008). Survival patterns of Dekkera bruxellensis in wines and inhibitory effect of sulphur dioxide. *International Journal of Food Microbiology, 121*(2), 201-207.

Bartowsky, E. J., Costello, P. J., & Chambers, P. J. (2015). Emerging trends in the application of malolactic fermentation. *Australian journal of grape and wine research*, *21*, 663-669.

Bartowsky, E., & A Henschke, P. (2004). The 'buttery' attribute of wine - Diacetyl - Desirability, spoilage and beyond*. International Journal of Food Microbiology 96*(3), 235-252.

Bartowsky, E. J., & Henschke, P. A. (2008). Acetic acid bacteria spoilage of bottled red wine - a *review. International Journal of Food Microbiology, 125*(1), 60-70.

Bell, S.-J., & A. Henschke, P. (2008). Implication of nitrogen nutrition for grapes fermentation and wine. *Australian Journal of Grape and Wine Research 11*(3), 242-295

Blanck, G., & Valade, M. (1989). Le fractionnement des moûts. *Le Vigneron champenois, 5*, 266-277.

Bornet, A., & Teissedre, P.-L. (2007). Chitosan, chitin-glucan and chitin effects on minerals (iron, lead, cadmium) and organic (Ochratoxin A) contaminants in wines. *European Food Research and Technology, 226*(4), 681-689.

Bosso, A., Panero, L., Guaita, M., & Marulli, C. (2001). La tecnica del delestage nella vinificazione del Montepulciano d'Abruzzo. *Enologo 37*, 87-96.

Bosso, A., Panero, L., Petrozziello, M., Follis, R., Motta, S., & Guaita, M. (2011). Influence of Submerged-Cap Vinification on Polyphenolic Composition and Volatile Compounds of Barbera Wines. *American Journal of Enology and Viticulture, 62*(4), 503-511.

Botezatu, A., Kotseridis, Y., Inglis, D., & Pickering, G. (2016). A survey of methoxypyrazines in wine. *Journal of Food, Agriculture & Environment, 14*(1), 24-29.

Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American Journal of Enology and Viticulture, 52*(2), 67-87.

Boulton, R., Singleton, V., Bisson, L., & Kunkee, R. (1999). Principles and practices of winemaking. New York: Chapman Hall, pp. 279-319.

Boursiquot, J.-M., Lacombe, T., Laucou, V., Julliard, S., Perrin, F. X., Lanier, N., Legrand, D., Meredith, C. P., & This, P. (2009). Parentage of Merlot and related winegrape cultivars of southwestern France: discovery of the missing link. *Australian Journal of Grape and Wine Research 15*(2), 144-155.

Cai, J., Zhu, B.-Q., Wang, Y.-H., Lu, L., Lan, Y.-B., Reeves, M. J., & Duan, C.-Q. (2014). Influence of pre-fermentation cold maceration treatment on aroma compounds of Cabernet Sauvignon wines fermented in different industrial scale fermenters. *Food Chemistry, 154*, 217-229.

Carbonneau A. 1985. The early selection of grapevine rootstock for resistance to drought conditions. *American Journal of Enology and Viticulture*, 36(3): 195-198.

Casassa, L. F., Beaver, C., Mireles, M., & Harbertson, J. (2012). Effect of extended maceration and ethanol concentration on the extraction and evolution of phenolics, colour components and sensory attributes of Merlot wines. *Australian Journal of Grape and Wine Research 19*(1):25-39.

Casassa, L. F., Bolcato, E. A., Sari, S. E., Fanzone, M. L., & Jofré, V. P. (2016). Combined effect of prefermentative cold soak and SO2 additions in Barbera D'Asti and Malbec wines:

Anthocyanin composition, chromatic and sensory properties. *LWT - Food Science and Technology, 66*, 134-142.

Casassa, L. F., & Harbertson, J. F. (2014). Extraction, evolution, and sensory impact of phenolic compounds during red wine maceration. *The Annual Review of Food Science and Technology, 5,* 83-109.

de Castilhos, M. B. M., Gómez-Alonso, S., García-Romero, E., Del Bianchi, V. L., & Hermosín-Gutiérrez, I. (2017). Isabel red wines produced from grape pre-drying and submerged cap winemaking: A phenolic and sensory approach. *LWT - Food Science and Technology, 81*, 58-66.

Chinnici, F., Natali, N., & Riponi, C. (2014). Efficacy of Chitosan in Inhibiting the Oxidation of (+)-Catechin in White Wine Model Solutions. *Journal of Agricultural and Food Chemistry, 62*(40), 9868-9875.

Chinnici, F., Sonni, F., Natali, N., Galassi, S., & Riponi, C. (2009). Colour features and pigment composition of Italian carbonic macerated red wines. *Food Chemistry, 113*(2), 651-657.

Christmann, M., & Freund, M. (2010). Advances in grape processing equipment, pp. 547-588.

Colangelo, D., Torchio, F., De Faveri, D. M., & Lambri, M. (2018). The use of chitosan as alternative to bentonite for wine fining: Effects on heat-stability, proteins, organic acids, colour, and volatile compounds in an aromatic white wine. *Food Chemistry, 264(1)*, 301-309.

Considine, J. A., & Frankish, E. (2014). Chapter 10 - Essential Analyses. In J. A. Considine & E. Frankish (Eds.), *A Complete Guide to Quality in Small-Scale Wine Making.* San Diego: Academic Press, pp. 137-154.

Dugelay, I., Gunata, Z., Sapis, J. C., Baumes, R., & Bayonove, C. (1993). Role of cinnamoyl esterase activities from enzyme preparations on the formation of volatile phenols during winemaking. *Journal of Agricultural and Food Chemistry, 41*(11), 2092-2096.

Du Toit, M., Engelbrecht, L., Lerm, E., & Krieger-Weber, S. (2011). Lactobacillus: the next generation of malolactic fermentation starter cultures—an overview. *Food and Bioprocess Technology, 4*(6), 876-906.

Du Toit, W. J., & Lambrechts, M. G. (2002). The enumeration and identification of acetic acid bacteria from South African red wine fermentations. *International Journal of Food Microbiology, 74*(1), 57-64.

Du Toit, W. J., Pretorius, I. S., & Lonvaud-Funel, A. (2005). The effect of sulphur dioxide and oxygen on the viability and culturability of a strain of Acetobacter pasteurianus and a strain

of Brettanomyces bruxellensisisolated from wine. *Journal of Applied Microbiology, 98*, 862-871.

Filipe-Ribeiro, L., Cosme, F., & Nunes, F. M. (2018). Reducing the negative sensory impact of volatile phenols in red wine with different chitosans: Effect of structure on efficiency. *Food Chemistry, 242*(1), 591-600.

Fornairon-Bonnefond, C., Demaretz, V., Rosenfeld, E., & Salmon, J. M. (2002). Oxygen addition and sterol synthesis in Saccharomyces cerevisiae during enological fermentation. *Journal of bioscience and bioengineering*, *93*(2), 176-182.

Freund, M., Seckler, J., & Jung, R. (2008). Grundsätzliches zu Pressprogrammen. *Der Deutsche Weinbau, 12*, 12-17.

Gobert, A., Tourdot-Maréchal, R., Sparrow, C., Morge, C., & Alexandre, H. (2019). Influence of nitrogen status in wine alcoholic fermentation. *Food Microbiology, 83*, 71-85.

Gómez-Plaza, E., & Cano-López, M. (2011). A review on micro-oxygenation of red wines: Claims, benefits and the underlying chemistry. *Food Chemistry, 125*(4), 1131-1140.

Guerrini, L., Angeloni, G., Baldi, F., & Parenti, A. (2016). Thermal effects of pump-overs during red wine fermentation. *Applied Thermal Engineering 112*, 621–626.

Gutiérrez-Gamboa, G., Díaz-Gálvez, I., & Moreno, Y. (2018). Effects of bub nodal position along the cane on bud fertility, yield component and bunch structure in 'Carménère' grapevines. *Chilean Journal of Agricultural Research. 78,* 580-586.

Gutiérrez-Gamboa, G., Pardo, C., & Moreno-Simunovic, Y. (2019). Effects on Berry Shrinkage in Vitis vinifera. L cv. 'Merlot' From Changes in Canopy/Root Ratio: A Preliminary Approach. *South African Journal of Enology and Viticulture, 40*(1). doi: https://doi.org/10.21548/40-1-2867

Hazelwood, L. A., Daran, J.-M., van Maris, A. J. A., Pronk, J. T., & Dickinson, J. R. (2008). The Ehrlich pathway for fusel alcohol production: a century of research on Saccharomyces cerevisiae metabolism. *Applied and Environmental Microbiology, 74*(8), 2259-2266.

Henschke, P. A. (1997) Stuck fermentation: causes, prevention and cure. In: Proceedings advances in juice clarification and yeast inoculation, Melbourne, Australia. Australian Society of Viticulture and Oenology, Adelaide, pp 30-41.

Heredia, F. J., Escudero-Gilete, M. L., Hernanz, D., Gordillo, B., Meléndez-Martínez, A. J., Vicario, I. M., & González-Miret, M. L. (2010). Influence of the refrigeration technique on the

colour and phenolic composition of Syrah red wines obtained by pre-fermentative cold maceration. *Food Chemistry, 118*(2), 377-383.

Hernandez-Orte, P., Bely, M., Cacho, J., & Ferreira, V. (2006). Impact of ammonium additions on volatile acidity, ethanol, and aromatic compound production by different Saccharomyces cerevisiae strains during fermentation in controlled synthetic media. *Australian Journal of Grape and Wine Research*, *12*(2), 150-160.

Jackson, R. S. (2008). Chapter 7. Fermentation. In: Wine Science (Third Edition). San Diego: Academic Press, pp. 333-401.

Jayani, R. S., Saxena, S., & Gupta, R. (2005). Microbial pectinolytic enzymes: a review. *Process Biochemistry*, *40*(9), 2931-2944.

Jégou, S., Hoang, D. A., Salmon, T., Williams, P., Oluwa, S., Vrigneau, C., Doco, T., & Marchal, R. (2017). Effect of grape juice press fractioning on polysaccharide and oligosaccharide compositions of Pinot meunier and Chardonnay Champagne base wines. *Food Chemistry, 232*, 49-59.

Keller, M. (2015). Chapter 8 - Living with Other Organisms. In M. Keller (Ed.), *The Science of Grapevines (Second Edition).* San Diego: Academic Press, pp. 343-367.

König, H., Unden, G., & Froehlich, J. (2009). Biology of Microorganisms on Grapes, in Must and in Wine. Springer: Berlin Heidelberg, Germany, pp. 3-60.

Lafon-Lafourcade, S., Geneix, C., & Ribéreau-Gayon, P. (1984). Inhibition of alcoholic fermentation of grape must by fatty acids produced by yeasts and their elimination by yeast ghosts. *Applied and Environmental Microbiology*, *47*(6), 1246-1249.

Lonvaud-Funel, A., Renouf, V., & Strehaiano, P. (2010). Microbiologie du vin : bases fondamentales et applications. Lavoisier, pp. 225-228.

Loureiro, V., & Malfeito-Ferreira, M. (2003). Spoilage yeasts in the wine industry. *International Journal of Food Microbiology, 86*(1), 23-50.

Malherbe, S., Bauer, F. F., & Du Toit, M. (2007). Understanding problem fermentations – A review. *South African Journal of Enology and Viticulture*, *28*(2), 169-186.

Marais, J. (2003). Effect of Different Wine-Making Techniques on the Composition and Quality of Pinotage Wine. II. Juice/Skin Mixing Practices. *South African Journal of Enology and Viticulture, 24(2),* 76-79.

Matthews, A., Grbin, P. R., & Jiranek, V. (2006). A survey of lactic acid bacteria for enzymes of interest to oenology. *Australian Journal of Grape and Wine Research*, *12*(3), 235-244.

Mendes-Ferreira, A., Mendes-Faia, A., & Leão, C. (2004). Growth and fermentation patterns of Saccharomyces cerevisiae under different ammonium concentrations and its implications in winemaking industry. *Journal of Applied Microbiology, 97*, 540-545.

Mihnea, M., González-SanJosé, M. L., Ortega-Heras, M., & Pérez-Magariño, S. (2015). A comparative study of the volatile content of Mencía wines obtained using different pre-fermentative maceration techniques. *LWT - Food Science and Technology, 64*(1), 32-41.

Moenne, I., Saa, P., Laurie, F., Pérez-Correa, J., & Agosin, E. (2014). Oxygen Incorporation and Dissolution During Industrial-Scale Red Wine Fermentations. *Food and Bioprocess Technology, 7*(9), 2627-2636.

Nishimura, K., Ohnishi, M., Masuda, M., Koga, K., & Mutsuyama, R. (1983). Reactions of wood components during maturation. J.R. Piggott (Ed.), Flavour of distilled beverages: Origin and development, Ellis Horwood Limited, England, pp. 241-255.

Nisiotou, A. A., Dourou, D., Filippousi, M.-E., Diamantea, E., Fragkoulis, P., Tassou, C., & Banilas, G. (2015). Genetic and Technological Characterisation of Vineyard- and Winery-Associated Lactic Acid Bacteria. *BioMed Research International,* Article ID 508254.

OIV. (2017). Compendium of International methods of wine and must analysis. International Organisation of Vine and Wine: Paris, France.

Ortega-Heras, M., Pérez-Magariño, S., & González-Sanjosé, M. L. (2012). Comparative study of the use of maceration enzymes and cold pre-fermentative maceration on phenolic and anthocyanic composition and colour of a Mencía red wine. *LWT - Food Science and Technology, 48*(1), 1-8.

Pace, C., Giacosa, S., Torchio, F., Río Segade, S., Cagnasso, E., & Rolle, L. (2014). Extraction kinetics of anthocyanins from skin to pulp during carbonic maceration of winegrape berries with different ripeness levels. *Food Chemistry, 165*, 77-84.

Pretorius, I. S. (2000). Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. *Yeast*, *16*(8), 675-729.

Renault, P., Coulon, J., de Revel, G., Barbe, J. C., & Bely, M. (2015). Increase of fruity aroma during mixed T. delbrueckii/S. cerevisiae wine fermentation is linked to specific esters enhancement. *International journal of food microbiology*, *207*, 40-48.

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B., & Lonvaud, A. (2006a). Chapter 13.5. Clarification. In: Handbook of enology: The microbiology of wine and vinifications, Vol. 1. Chichester: Wiley, pp. 421-428.

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B. & Lonvaud, A. (2006b). Chapter 12.3. Filling Vats. In: Handbook of enology: The microbiology of wine and vinifications, Vol. 1. Chichester: Wiley, pp. 334-336.

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B. & Lonvaud, A. (2006c). Chapter 12.6. Running Off and Pressing. In: Handbook of enology: The microbiology of wine and vinifications, Vol. 1. Chichester: Wiley, pp. 358-367.

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B. & Lonvaud, A. (2006d). Chapter 3.4. Nutrition Requirements. In: Handbook of enology: The microbiology of wine and vinifications, Vol. 1. Chichester: Wiley, pp. 84-89.

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B. & Lonvaud, A. (2006e). Chapter 8.5. Practical consequences: The state of sulfur dioxide in wines. In: Handbook of enology: The microbiology of wine and vinifications, Vol. 1. Chichester: Wiley, pp. 206-209.

Sablayrolles, J. M. (2009). Control of alcoholic fermentation in winemaking: Current situation and prospect. *Food Research International, 42*(4), 418-424.

Sacchi, K. L., Bisson, L., & Adams, D. O. (2005). A Review of the Effect of Winemaking Techniques on Phenolic Extraction in Red Wines. *American Journal of Enology Viticulture, 56*, 197-206.

Sala, C., Busto, O., Guasch, J., & Zamora, F. (2004). Influence of vine training and sunlight exposure on the 3-alkyl-2-methoxypyrazines content in musts and wines from the Vitis vinifera variety Cabernet Sauvignon. *Journal of agricultural and food chemistry*, *52*(11), 3492-3497.

Silva, I., Campos, F. M., Hogg, T., & Couto, J. A. (2011). Factors influencing the production of volatile phenols by wine lactic acid bacteria. *International Journal of Food Microbiology, 145*(2-3), 471-475.

Souquet, J.-M., Cheynier, V., & Moutounet, M. (2000). Les proanthocyanidines du raisin. Bulletin de l'OIV, 73, 601-609.

Spasic, A. M. (2018). Chapter 1 - Introduction. In A. M. Spasic (Ed.), Interface Science and Technology, Elsevier, pp. 1-25.

Spranger, I., Cristina Clímaco, M., Sun, B., Eiriz, N., Fortunato, C., Nunes, A., Conceição Leandro, M., Luísa Avelar, M., & Pedro Belchior, A. (2004). Differentiation of red winemaking technologies by phenolic and volatile composition. *Analytica Chimica Acta, 513*(1), 151-161.

Suárez, R., Suárez-Lepe, J. A., Morata, A., & Calderón, F. (2007). The production of ethylphenols in wine by yeasts of the genera Brettanomyces and Dekkera: A review. *Food Chemistry, 102*(1), 10-21.

Sudraud, P., & Chauvet, S. (1985). Activité anti-levure de l'anhydride sulfureux moléculaire. *Connaissance de la Vigne et du Vin, 19,* 31-40.

Sun, B., Spranger, I., Roque-Do-Vale, F., Leandro, C., & Belchior, P. (2001). Effect of Different Winemaking Technologies on Phenolic Composition in Tinta Miùda red wines. *Journal of Agricultural and Food Chemistry, 49*, 5809-5816.

Tesniere, C., & Flanzy, C. (2011). Chapter 1 - Carbonic Maceration Wines: Characteristics and Winemaking Process. In R. S. Jackson (Ed.), *Advances in Food and Nutrition Research*, *63,* 1-15, Academic Press.

Torrea, D., Varela, C., Ugliano, M., Ancin-Azpilicueta, C., Leigh Francis, I., & Henschke, P. A. (2011). Comparison of inorganic and organic nitrogen supplementation of grape juice – Effect on volatile composition and aroma profile of a Chardonnay wine fermented with Saccharomyces cerevisiae yeast. *Food Chemistry, 127*(3), 1072-1083.

Tubia, I., Prasad, K., Perez-Lorenzo, E., Abadin, C., Zumarraga, M., Oyanguren, Barbero, F., Paredes, J., & Arana, S. (2018). Beverage spoilage yeast detection methods and control technologies: A review of Brettanomyces. *International Journal of Food Microbiology, 283*(1), 65-76.

Valera, M. J., Sainz, F., Mas, A., & Torija, M. (2017). Effect of chitosan and SO2 on viability of Acetobacter strains in wine. *International Journal of Food Microbiology, 246*(1), 1-4.

Zoecklein, B. W., Birkenmaier, S. S., & Pélanne, L. M. (2009). Impact of délestage with partial seed removal. *Practical Winery & Vineyard. 30*(2), 34-52.