Filipa Vieira Carreira

Comparative study of two tanks of Syrah wine with same harvest fermented by two different *Saccharomyces cerevisiae* strains

Master thesis in Food Safety, supervised by Master Marjorie Spurr and Professor Doctor Fernando Ramos, submitted to the Faculty of Pharmacy, University of Coimbra

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To my friend G.

"O começo de todas as ciências é o espanto de as coisas serem o que são"

- Aristóteles

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Abstract

Wine has an important role in human diet, not only due to its unique organoleptic characteristics and therapeutic properties, but also for being an important component of the Mediterranean diet.

Currently, many biotechnology institutes and laboratories are developing studies to create yeast strains with oenological characteristics for winemaking process to obtain a quality wines. These characteristics may include fermentative rate, tolerance to alcohol, acetic acid production and thermic tolerance.

ICV D80® and FERMOL® SUPER 16 are two different yeast strains of *Saccharomyces cerevisiae* with different oenological characteristics produced in independent laboratories. The aim of this work is to compare the effect of these two dried commercial yeast strains, in a chemical and fermentative process perspective in *Enclos des Anges* company. The Syrah grape berries were used in this red winemaking process, obtained from the 2016 harvest. These analyses were performed in different stages of the winemaking process, mainly before, during and after fermentations. After fermentations, in ageing stage, the routine and reference analysis were performed to control the chemical composition of Syrah wine. The compounds identified included alcohol strength, reducing sugars, total acidity, volatile acidity, pH, free SO_2, total SO_2, malic and lactic acids.

Comparing both yeast, the results obtained were very similar in the performed analysis. Nevertheless, FERMOL® SUPER 16 yeast may have demonstrated a higher efficiency in the majority of the parameters observed, namely volumetric mass and temperature during alcoholic fermentation, and alcohol strength, volatile acidity and free and total SO_2 after fermentations.

Keywords: chemical, fermentations, food safety, FERMOL® SUPER 16 yeast, ICV D80® yeast strain, red wine, *Saccharomyces cerevisiae*, Syrah, winemaking process

Resumo

O vinho é uma bebida importante na dieta humana, não só devido às suas características organoléticas e propriedades terapêuticas, mas também por ser um elemento integrante da dieta mediterrânea.

Muitos institutos e laboratórios do ramo da biotecnologia têm desenvolvido estudos com o intuito de produzirem estirpes de levedura com características enológicas que fomentam o processo de vinificação para obter vinhos de qualidade. Nestas características podem constar a taxa fermentativa, a tolerância ao álcool, a produção de ácido acético e a tolerância térmica. A ICV D80® e a FERMOL® SUPER 16 são duas estirpes de leveduras de *Saccharomyces cerevisiae* com diferentes características enológicas provenientes de laboratórios distintos. O presente trabalho teve como objetivo a comparação de duas estirpes de leveduras comerciais secas ativas, ICV D80® e FERMOL® SUPER 16, na perspetiva dos processos químicos e fermentativos na empresa *Enclos des Anges*. Os cachos da variedade Syrah foram utilizados no processo de vinificação do vinho tinto, obtidas na colheita de 2016. As análises foram realizadas em diferentes fases do processo de vinificação: antes, durante e após as fermentações. Após as fermentações, nomeadamente na maturação do vinho, as análises de rotina e referência foram realizadas para controlar a composição química do vinho Syrah. Os compostos identificados abrangeram o teor alcoólico, os açúcares redutores, a acidez total, a acidez volátil, o pH, o SO_2 livre, o SO_2 total, o ácido málico e lático.

Comparando ambas as estirpes de levedura, os resultados obtidos foram muito semelhantes nas análises efetuadas. Contudo, a levedura FERMOL® SUPER 16 parece ter demonstrado uma maior eficiência na maioria dos parâmetros observados, nomeadamente na massa volúmica e na temperatura durante a fermentação alcoólica e no teor alcoólico, na acidez volátil e no SO_2 livre e total depois das fermentações.

Palavras-chave: fermentações, levedura FERMOL® SUPER 16, levedura ICV D80®, *Saccharomyces cerevisiae*, segurança alimentar, Syrah, vinho tinto, vinificação

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List of abbreviations

AF – Alcohol fermentation

AS – Alcohol strength

BTU – Basic *Terroir* Units

DAP – Di-ammonium phosphate

EC – European Commission

FTIR – Fourier Transform Infrared Spectroscopy

HACCP – Hazard analysis and critical control points

ICV D80® – *Institute Coopératif du Vin* D80®

IFV – French Institute of Vine and Wine

IHEV – Institute for Higher Education in Vine and Wine Sciences

INRA – French National Institute for Agricultural Research

LD_{50} – Lethal dose 50%

meq – milliequivalents

mhl – million hectolitres

MLF – Malolactic fermentation

OIV – International Organisation of Vine and Wine

PAC – Potential alcohol content

TA – Total acidity

TCA – Tricarboxylic acid cycle

USA – United Sates of America

VA – Volatile acidity

Vol. – volume

Chapter 1
Introduction

1. Introduction

Since prehistory until current times that the historical geography of viticultures and the wine business have a great impact in humans' lives. By the fourteenth century wine had become an essential part of the culture of the dominant classes of northern and western Europe, because Greeks and Romans had colonised centuries earlier most of the Mediterranean area (Unwin, 1996). According to the International Organisation of Vine and Wine (OIV, 2016a), wine is the drink resulting exclusively from the partial or complete alcoholic fermentation of fresh grapes, whether crushed or not, or of grape must. The global wine production in 2016, excluding juice and musts, was likely to reach 259.5 mhl and in France was 41.9 mhl (OIV, 2016b).

Wine has a high impact in several areas, such as economic, social, political, ideological and commercial areas.

For hundreds of years, France claimed the reputation of being the world's greatest producer of wines, although the wine industry had been an important sector in many Mediterranean countries (Meneses *et al.*, 2016). Nowadays, that reputation is being rivalled by other wine-growing nations on four continents, and the French wine industry is facing new challenges, such as producing new styles of wines, improving winegrowing techniques and accommodating new winemaking equipment while respecting centuries of winegrowing tradition. Also, the customers' choice and trade is a concern of winemakers and they begin to follow customer trends. These trends include not only the way of cultivation and the variety of grapevine but also, the choice, the shape and the colour of label and stoppers.

In addition, wine is part of a healthy lifestyle due to its bioactive properties and its intake may be part of a balanced dietary pattern (Fernández-Mar *et al.*, 2012).

French Paradox based on epidemiological studies shows that people living in certain regions of France who consumed moderate quantities of wine had a lower incidence of coronary disease than those living in other countries with similar dietary habits but who did not consume alcoholic drinks in the same quantities (Miller *et al.*, 1990). According to Vaquero *et al.* (2007) the consumption of wine, particularly red wine, has an influence on the reduction of cardiovascular diseases, the reduction of the risk of developing oncologic diseases, anti-inflammatory activity, antimicrobial activity, renal and neurological protection.

All these reasons make the study of wine and its production relevant. So, there are many researchers improving the scientific wine domain in many different areas, such as, viticulture,

oenology, microbiology, food safety, sensorial evaluation, chemistry, genetic, physic, biotechnology, marketing and wine selling.

The microbial perspective is very relevant, because wine depends on yeasts and the oenologist wants to find the best combination between winemaking process and wine characteristics. Thus, it is relevant to search the ideal yeast, therefore, the development of this study was important.

Two different *Saccharomyces cerevisiae* yeast strains in two tanks of Syrah wine with same harvest were studied. Several analyses along different stages of fermentation were performed in three places.

This work was carried out in France (Figure 1):

- on the French island of Corsica located in East Mediterranean sea: in Calvi *Enclos des Anges* company and in Bastia Departmental Laboratory of Analysis;
- South France mainland: in Institute for Higher Education in Vine and Wine Sciences (IHEV) in Montpellier.

Figure 1 – The three different places where this work was performed: Montpellier, Bastia and Calvi. Source: Google Earth

This study was performed in different stages: before, during and after fermentation processes. Before and during fermentation process, the study was controlled in *Enclos des Anges* company. The stage after fermentation was performed in two different places: Bastia Department Laboratory and Montpellier SupAgro. In Bastia Department Laboratory the routine analysis

were performed using a FTIR equipment with *BACCHUS* Acquisition Software. In IHEV the references analysis were performed following the Compendium of International Methods of Wine and Must Analysis of International Organisation of Vine and Wine (OIV).

1.1. Winemaking and Viticulture Company: *Enclos des Anges*

The *Enclos des Anges* company (Figure 2) is located in Corsica, more precisely in Calvi (northwest of the island), and was started by Richard and Marjorie Spurr, in 2007, from vineyards and an existent wine cellar. Since then, they have restructured the wine cellar and the vineyards through their viticulture skills and oenological knowledge. They work in several vineyards with different grapes varieties: Niellucciu, Syrah, Grenache, Vermentinu and Sciacarello. They produce different types of wine, including red, white and rosé, following vines and production methods which satisfy regulations and laws. These regulations means controlled origin, AOP - *Appellation d'Origine Protégée*, and guarantees that the wine and other food products, are produced in a specific region.

ENCLOS DES ANGES

Figure 2 – *Enclos des Anges* logo

Currently, the company has a production of 25.000 bottles/year, particularly white and red wines with 11.000 bottles/year and rosé wines with 3.000 bottles/year. These crops are produced from 14 ha of vineyards with a remaining 6 ha on which young vines are growing, that are not yet productive. Nowadays, the company has created other label, the *Semper Fidelis* label (Figure 3). The company has reached different markets, such as wine houses of the Corsica regions, and restaurants and hotels in mainland of France and United States of America, Belgium and Switzerland. They also have a cellar shop.

Figure 3 – Different labels of *Enclos des Anges* company

1.2. *Terroir*

Geographic factors and other environmental factors, including climate and soil, contribute to the identity of a wine from a given region – *terroir* (Hénaff *et. al.*, 2016).

According to Morlat (2001), the *Unité Terroir de Base* (UTB) corresponds to an area that is sufficiently homogeneous in terms of the functioning of the *terroir*/vine/wine system and that also has sufficient surface area for viticulture, agronomic, pedology, climate and commercial valorization.

The *terroir* effect results from the combination of several factors related to the natural environment, mainly geology, morphology, soil and climate, agronomic factors, such as rootstocks and grape varieties, and human factors, like cultural and oenological practices (Morlat, 2001; Van Leeuwen and Chery, 2001; Morlat and Meinen, 2003) (Figure 4).

Figure 4 – *Terroir* viticulture

According to the term *terroir*, *Enclos des Anges* company and this study, the vineyards are located in windy region with east facing sloping plots, grown in the goblet method. Soil types are degraded granite soils, which are acidic, and the climate is Mediterranean (see Attachment 1).

1.3. Grapevine variety Syrah

Syrah has been known in the Rhône Valley of France and its parentage determined through DNA testing to be a cross between two French varieties, *Dureza* and *Mondeuse Blanche* (Christensen, 2003). Syrah is one of the oldest cultivated varieties and has a good response to the modification of grapevine cycle (Lima *et al.*, 2015; Mota *et al.*, 2012). According to Kerridge and Antcliff (1999) Syrah is a vigorous variety with a spreading habit of growth and with unique features (Figure 5).

Figure 5 – Syrah grapevine and grape berries.
Source: David McSpadden in http://www.ecobalade.fr/espece/vigne-cepage-Syrah

Botanically, it has medium, 5-lobed leaves, somewhat rough and undulating, with tufted hairs on the lower surface (Sereno, 2009). The bunches are characteristically long and cylindrical with long stalks, rather loose, with small to medium oval berries which tend to wilt as soon as they are ripe, becoming more difficult to harvest mechanically (Kerridge and Antcliff, 1999). It has a complex aroma that includes fruity flavours of blackberry and plum balanced out with spicy-peppery and earthy-leathery notes (Henderson and Rex, 2011).

1.4. Red wine and its composition

In case of red wines, they are obtained by the alcoholic fermentation of musts in the presence of the solid parts of the berry - skins and seeds (Ribéreau-Gayon, 2006). The sweetness, alcohol content, carbon dioxide content, colour, grape variety, fermentation, maturation process and geographic origin are characteristics that classify the wine as red, white and rosé (Jackson, 2009). Moreover, wines can be distinguished by the geographic location of vineyards, variations in the same vineyard, different viticultural practices, winemaking processes and ageing techniques (Dennis *et. al.* 2012).

Wine composition depends greatly on grape variety but also on viticulture management that interferes with grapevine metabolism and on the winemaking processes, namely during fermentation as a function of the yeasts and bacteria present and their respective metabolisms (Bejerano and Zapeter 2013; Keyzers and Boss 2010; Boss *et al.* 2014). This drink remains a very complex medium and its composition depends both on the grapes' components and the interactions between grapes that occur during wine production (Rosado, 2013). These interactions may have implications not only in the chemical stability of the wine but also in the sensorial stability (Rosado, 2013). However, there are many components that have not been identified and their contribution to the final product is unknown (Curvelo-Garcia, 1988).

In Table 1, there is an average composition of red wine, based on Labruyere *et al.* (2015).

Table 1 – Chemical composition of red wine in weight percent. Adapted from Labruyere *et al.* 2015

	Wine composition (%)
Water	80.5
Sugars (simple, complex)	0.2
Ethyl alcohol	12
Organic acids (tartaric, malic, citric acid)	0.4
Minerals (potassium, phosphorus, sulphur)	0.2
Phenols (tannins, pigments)	0.1
Nitrogen	0.03
Esters, aldehydes, higher alcohols	0.03
Non-specified	6.54

1.4.1. Water

Water is the major component of wine and its most organic functional groups are based on the double covalent bond or on bonds with oxygen, nitrogen and sulphur (Jackson, 2008). However, water plays a critical role due to its specific characteristics (Genc *et al.*, 2017):

- Establishes the basic characteristics of wine;
- Governs the basic flow characteristics;
- Is a relevant component in many chemical reactions involved in grape growth, juice fermentation and wine ageing.

1.4.2. Sugars

Sugars are a category of carbohydrates and they can be divided up into simple sugars and complex sugars. Grape sugar content depends on the species, variety, maturity and health of the fruit (Jackson, 2008).
Glucose and fructose (Figure 6) are the two main simple sugars in must wine and they are critical to yeasts' growth and metabolism (Breinig *et al.*, 2006).

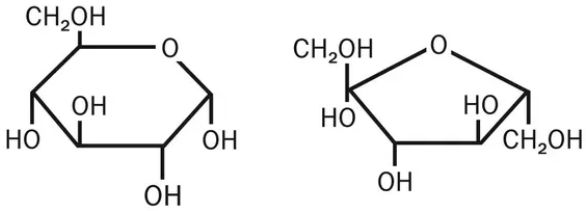
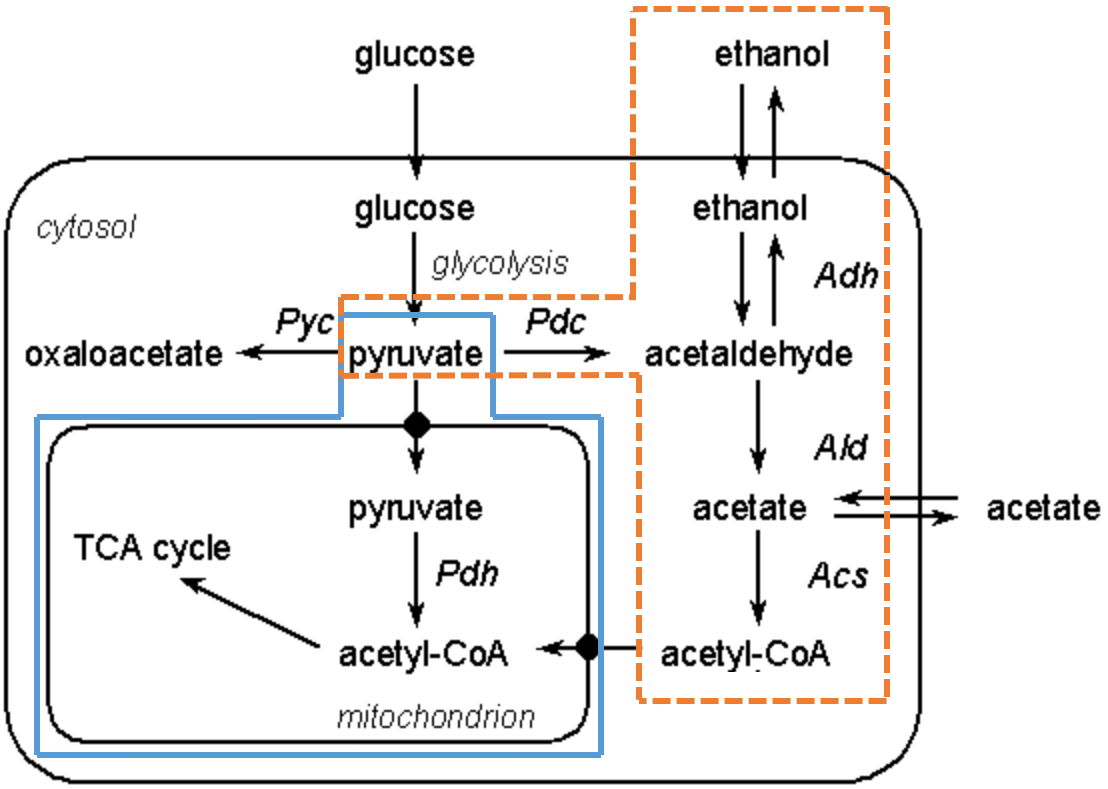


Figure 6 – Structural representation of the most abundant hexoses in the must.
On the left: the glucose; on the right: the fructose.

1.4.2.1. Sugar degradation pathways in yeast

Depending on aerobic or anaerobic conditions, yeasts can degrade sugars using two metabolic pathways: respiration or alcoholic fermentation, respectively. These two processes begin in the same way, sharing the common trunk of glycolysis (Jackson, 2009) (Figure 7).



Legend: Pyc - pyruvate carboxylase; **Pdc** - pyruvate decarboxylase; **Adh** - alcohol dehydrogenase; **Ald** - acetaldehyde dehydrogenase; **Acs** - acetyl-coenzyme A synthetase; **Pdh** - pyruvate-dehydrogenase

Figure 7 – Yeast energy metabolism with glycolysis, respiration (blue) and fermentation (orange) pathways. Source: Flikweert (1999)

Sugars are converted into pyruvic acid during glycolysis in cell cytosol which results in the production of two molecules of pyruvate and ATP per glucose. When oxygen (O_2) is available, pyruvic acid enters a series of chemical reactions, known as the Krebs cycle or Tricarboxylic acid (TCA) cycle, and proceeds to the respiratory chain in mitochondria (Alba-Lois and Segal-Kischinevzky, 2010). As a result of respiration, cells produce 36-38 molecules of ATP for each molecule of glucose oxidized. When O_2 is limited, pyruvic acid can be converted into ethanol and carbon dioxide (CO_2) through the alcoholic fermentation pathway with yeast microorganism, such as *Saccharomyces cerevisiae* (Ribereau-Gayon *et al.*, 2006). In addition, there is another phenomenon that can be observed in the fermentation process – the Crabtree effect. The Crabtree effect describes the phenomenon whereby the yeast, *Saccharomyces cerevisiae*, produces ethanol in aerobic conditions and high external glucose concentrations rather than producing biomass via the TCA cycle, the usual process occurring aerobically in most yeasts (Pfeiffer and Morley, 2014).

1.4.2.2. Reducing sugars

Unfermented sugars, mainly pentose sugars, are collectively named residual sugars and their content is generally less than 2.0 g/l. Although residual sugars, such arabinose, rhamnose and xylose, are obviously important to the sweetness of wine, fermentable sugars in grapes are absolutely essential for fermentation (Ribereau-Gayon *et al.*, 2006). In addition, reducing sugars interact with other components, such as higher alcohols, fatty acid, esters and aldehydes. These give different wines much of their aromatic character. These sugars must be controlled in final process of fermentation and in ageing stage, through routine analysis, because undesirable bacteria and yeasts can develop and therefore spoil the wine (Jackson, 2008).

1.4.3. Ethyl alcohol

Ethyl alcohol, or ethanol, is one of, alongside with CO_2 and heat, the final product of alcohol fermentation due to the fermentation of sugars by yeasts. Ethyl alcohol is a volatile, flammable, antiseptic, colorless liquid with a slight characteristic odor (Kotz *et al.*, 2017).
The winemaking parameters that increase ethanol production by yeasts are well known, such as high pH, elevated fermentation temperature, and aeration (Ribereau-Gayon *et al.*, 2006).

1.4.4. Nitrogen

Nitrogen is part of the ammonium cation, of amino acids, of polypeptides and of proteins. The grape nitrogen concentration depends on variety, rootstock, environment and growing conditions, especially nitrogen fertilization (Ribereau-Gayon *et al.*, 2006). Nitrogen is needed for yeast growth in grape must and it accelerates fermentation in the early stages by increasing yeast population and improving the quality of cell membranes' permeability (Ribereau-Gayon *et al.*, 2006).
However, the extent and frequency of nitrogen deficiencies have been demonstrated in recent years, due to changes in vineyard management techniques. Therefore, the wineries started putting nitrogen supply in winemaking process (Jackson, 2008).
Yeasts contain a series of transport molecules in cell membrane responsible for the uptake of nitrogen sources from the medium and some of which also act as receptors signalling the

vailability of their substrate to the interior of the cell (André, 1995; Holsbeeks *et al.*, 2004). This receptors allow cells to have a better nitrogen source available, which can be imported through specific permeases, mainly Mep1 and Mep2 for ammonia, Gap1p for arginine and Gnp1 for glutamine (Figure 8) (Garrett, 2008).

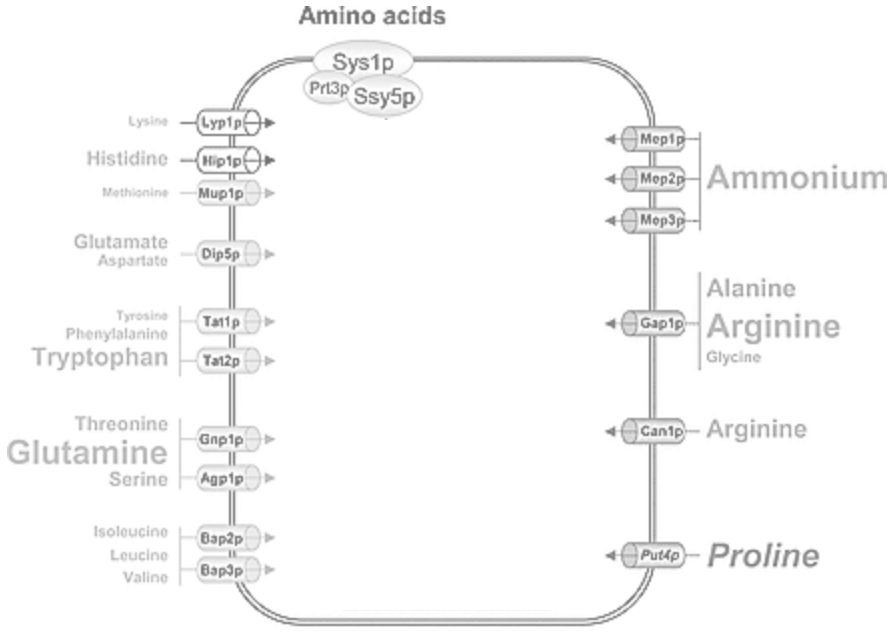


Figure 8 – Majority nitrogen source transporters in yeast. Adapted from Crépin *et. al.*, 2012

Di-ammonium phosphate (DAP) was the nitrogen supply used in *Enclos des Anges* winery. According to Commission Regulation (EC) n°606/2009 the limits on application of DAP is1g/l. Adding nitrogen to musts containing insufficient levels is extremely useful in achieving good fermentation kinetics and it goes inside of the yeast cells easily (Ribereau-Gayon *et al.*, 2006). According to Henschke and Jiranek (1992), with different *S. cerevisiae* strains, temperature increases nitrogen assimilation.

1.4.5. Organic acids

Most of the energy comes from the assimilation of numerous organic substrates, sugars, amino acids and organic acids (Jackson, 2008). The four principal organic acids in all winemaking process are tartaric, malic and lactic acid, depending on malolactic fermentation taking place,

and citric acid. However, the tartaric, malic and citric acids found in grapevine ripening comes from all parts of the grapevine (Blouin and Guimberteau, 2000).

Oenococcus oeni bacteria have an important role in degrading the malic acid, into lactic acid, and citric acid which are evidently the source of many organoleptic changes noted in the final product. However, these bacteria produce acetic acid, which in high quantities can be undesirable in wine (Ribereau-Gayon *et al.*, 2006).

1.4.5.1. Tartaric acid

The tartaric acid in the grape berry is found in a high amount, compared with other plant species, in L (+) isomer (Peynaud, 1947, Champanagol 1984, Favarel, 1994). It is a strong acid that interferes directly with pH and it is resistant to the cell respiration (Rizzon and Sganzerla, 2007). The tartaric acid content in the must ranges from 3-9 g/l, according to the grape variety and the conditions of grape production, especially the availability of water (Blouin and Guimberteau, 2000).

The presence of wine residues is usually identified by the presence of tartaric acid residues. Tartaric acid is metabolized by few microorganisms and it is characteristic of *V. vinifera* specie, essentially from pulp grape (Jackson, 2008). During the ageing process, undissolved tartates crystallize with potassium and calcium and precipitate and this process can be accelerated by cooling (0°C) and addition of potassium tartate crystals. This partially occurs due to the conversion of the natural (L form) of tartaric acid to the D isomer (Figure 9) (Jackson, 2008).

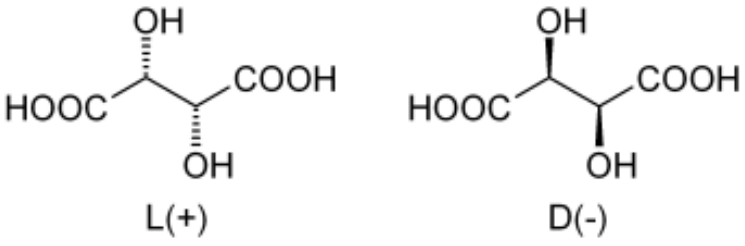


Figure 9 – Tartaric acid. On the left: L isomer; On the right: D isomer.

1.4.5.2. Malic Acid

The natural malic acid of the grape is the L (-) isomer (Peynaud, 1947; Champagnol, 1984; Favarel, 1994). It is one of the most widespread organic acids in nature, predominating in a lot of fruits (Rizzon and Sganzerla, 2007). It is considered a weak acid and has a low resistance to oxidative respiration (Rizzon and Sganzerla, 2007). In the grapevine, the synthesis of malic acid results from a secondary reaction of photosynthesis, occurring mainly in the adult leaves of the grapevine (Rizzon and Sganzerla, 2007).

Malic acid may constitute about half the total acidity of grapes and wine. Its concentration in the fruit tends to decrease as grapes mature, especially during hot periods at the end of the season, unlike tartaric acid (Ribereau-Gayon *et al.*, 2006).

The transformation of malic acid in lactic acid is the most important phenomenon of the malolactic fermentation phase by lactic acid bacteria (Figure 10).

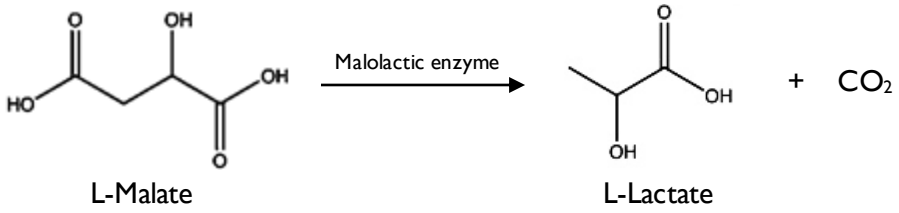


Figure 10 – Malolactic fermentation
Adapted from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3669155/figure/F1/

1.4.5.3. Citric acid

The presence of citric acid (Figure 11) in the vine is small, and found more in the subterranean roots than in the air. In the roots it appears with the reaction of the carbonates of the soil with the carbon dioxide, whereas in the aerial parts it arises by the action of enzymes on the malic acid (Borges, 2008).

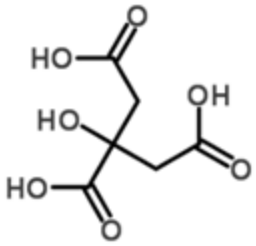


Figure 11 – Citric acid

Certain lactic acid bacteria, such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Oenococcus oeni* and *Leuconostoc mesenteroides,* degrade citric acid. Its degradation begins at the same time as malic acid degradation, but it is much slower. Moreover, the fermentation of citric acid is enhanced by the presence of phenolic compounds (Rozès *et al.*, 2003).

1.4.6. Minerals

Many mineral elements, such as potassium, phosphorus and sulphur, are found in grapes and wine and its mineral concentration reflects the uptake characteristics of the rootstock and the climatic influences on the rate of transpiration (Jackson, 2009). According to Fregoni (1977) differences in wine quality results from different minerals present in vineyard soils (see Attachment 1).

1.4.7. Phenols

Tannins and anthocyanins pigments (Figure 12) are responsible for the colour of red grapes and wines and constitute the two most abundant classes of phenolics in the berry skins (Cheynier *et al.*, 2006).

Both anthocyanins and tannins are partly extracted from grape skins during winemaking and can undergo structural transformations through many reactions with significant influence on wine sensory characteristics, as they are involved in astringency, bitterness, color intensity, and color stability (Fournand *et al.*, 2006).

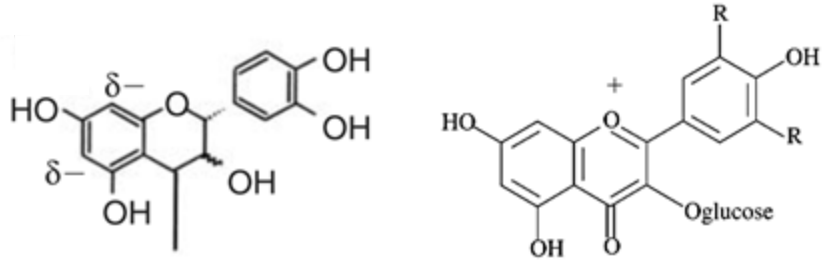


Figure 12 – On the left: tannin; On the right: anthocyanins. Source: Jackson, 2008

1.4.8. Esters, aldehydes, higher alcohols (volatile)

Esters, aldehydes and higher alcohols are compounds present in grape berry skin and pulp, in the form of sugar-bound precursors, and they also come from yeast metabolism (see topic 1.6.2.) (Ribereau-Gayon *et al.*, 2006).

They are classified as aromatic compounds characterizing the wine in its different odor, such as flowers, fruits, seeds, leaves, woods and roots (Jackson, 2009).

1.5. Red winemaking process

Various approaches can be, and are, taken in consideration in the production of red wine and many different processes may be used.

According to Antonelli *et al.* (2010) classic steps in red winemaking are:

- mechanical harvest treatments - crushing, destemming and tank filling;
- vatting - primary alcoholic fermentation and maceration;
- draining - separation of wine and pomace by dejuicing and pressing;
- final fermentations - exhaustion of the last grams of sugar by alcoholic fermentation and malolactic fermentation.

However, other techniques have been developed and certain operations have changed to reach a level of automation. Furthermore, other winemaking products can be added within certain quantities according to laws and regulations.

In *Enclos des Anges*, the process starts with the grapes' harvest in September, usually in the beginning of the month, and it is concluded one and a half year later, usually in March, with boxes' storage. The system consists mainly of ten parts, namely the harvesting grapes, the transport, the reception, the destemmer, the alcoholic fermentation, the malolactic fermentation, the crush, the ageing tank, the filtration, bottling, labelling and box storage (Figure 13).

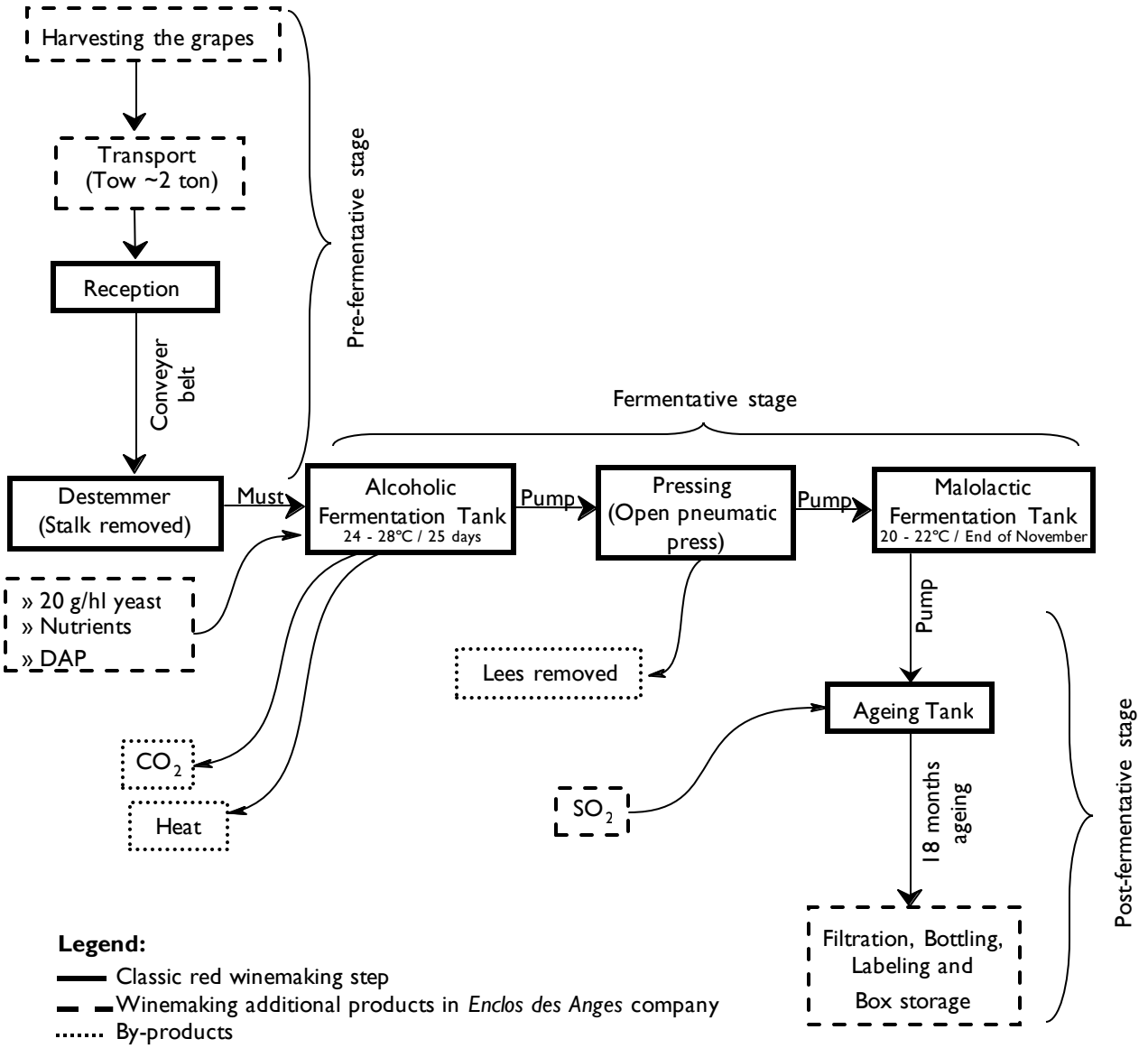


Figure 13 – Syrah red winemaking process in *Enclos des Anges* company

Biochemical and mechanic operations occur along pre-fermentative, fermentative and post-fermentative stages.

After the manual harvest, in reception, the grapes are moved with conveyer belt until destemmer machine where the grapes are separated from the stalks and subsequently broken to allow for the fermentation of the juice. Alcoholic fermentation occurs while the juice is in contact with skins for an extended maceration to further develop the wine character. The separated stalks are the first waste of the process. In the next step, the must goes to an open tank with temperature control where alcoholic fermentation takes place. Alcoholic fermentation is initially activated by active dried yeast strains inoculation where yeast primarily turns the sugar into alcohol, heat and carbon dioxide (CO_2). These two final by-products are emitted to the cellar's atmosphere. This reaction is exothermic and fermentation must be carried out under controlled temperature where the optimal range is 25–30°C during 25 days. Otherwise, in this final stage, the winemakers increase the temperature to extract phenolic compounds.

The temperature of the must can also be adjusted to enhance or reduce the rate of the phenolic compounds of the grape-tannins, colouring agents, mainly anthocyanins, and flavour compounds from the grape skins, seeds and stems into the must. During the alcoholic fermentation, and depending on the needs of the environment tank, a nutritive complex (NUTRICELL FINISH) and di-ammonium posphate (DAP) (ENOVIT®) are added. Furthermore, two processes occur in this stage: pump-over and devatting (see Topic 3.3).

Malolactic fermentation is carried out by lactic acid bacteria that turns the malic acid into the softer lactic acid with the temperature below 22°C. After first and secondary fermentation wine is left for a period for maturation with the addition of 2-3g/hl sulphur dioxide (SO_2). This compound inhibits wild and spoilage yeasts and unwanted bacteria and helps preventing oxidation and preserves fruity flavour and freshness in wine. Before ageing period in stainless steel tanks, the mixture follows the crush and transfer stage in an open pneumatic press by a must pump and the lees are separated from wine. During this stage the temperature is controlled, which in the winter is around room temperature without any auxiliary equipment and in the summer is around 18°C through a cooling system. The tartaric stabilization is obtained over the 18 month ageing process, the precipitation of the unstable tartaric salts is considered finished in this lapse of time. Completed the 18 months ageing, the wine production process ends up with the bottling facility, with wine pumped through a filter for clarifying.

1.6. Wine yeasts

Wine is the product of complex interactions among yeast, bacteria and other fungi that begin in vineyards and continue with the fermentation process until packaging (Fleet, 2003). Microbial communities of the grape berry, the winery environment and the winery equipment moderate the growth of spoilage and mycotoxigenic fungi and the species and yeast strains contribute to alcoholic fermentation (Elmaci *et al.*, 2014; Romano *et al.*, 2003). Some of non-*Saccharomyces* species from various microbial communities (such as *Hanseniaspora*, *Candida*, *Pichia* and *Metschnikowia*) initiate spontaneous alcoholic fermentation of the juice, but are very soon overtaken by the growth of *Saccharomyces cerevisiae* that dominates from the mid to final stages of the process (Albergaria and Arneborg, 2016) (Figure 14). *Saccharomyces cerevisiae* is the main microorganism responsible for the fermentation of wine, due to its resistance to different hostile factors (Alonso-del-Real *et al.*, 2017; Rosado, 2013):

- Resistance to high concentrations of sugars and the ability to ferment almost all the sugars that make up the must;
- Resistance to high concentrations of SO_2;
- Resistance to low pH values;
- Resistance to high ethanol contents.

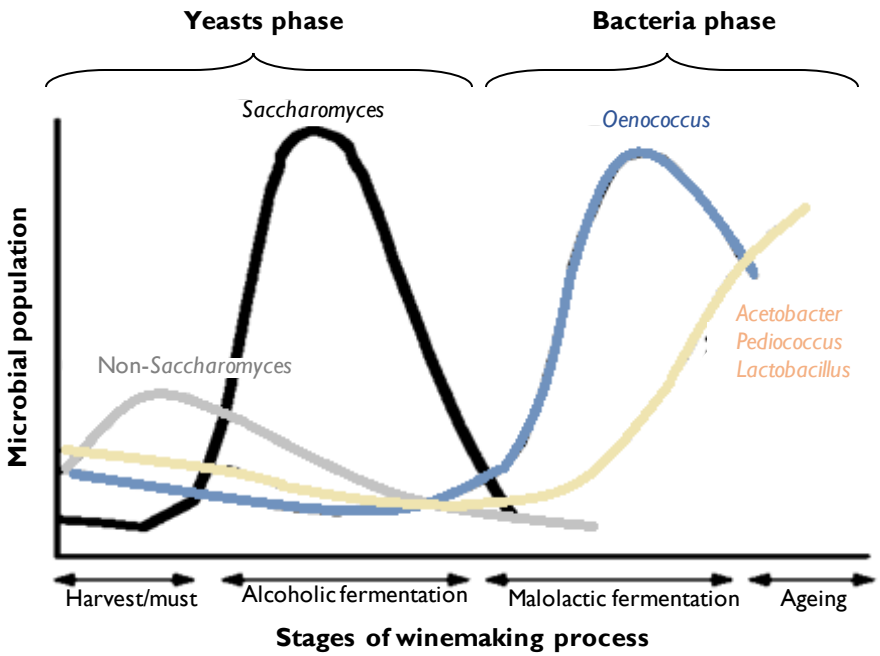


Figure 14 – General microbial ecology during winemaking process
Adapted from Osborne and Edwards (2005)

S. cerevisiae is an unicellular fungi belonging to the class Ascomycetes which multiply either asexually by vegetative multiplication, budding, or sexually by forming ascospores (Skoneczna *et al.*, 2015). The size of yeasts is 4-8 µm in width and 7-12 µm in length (Johnston *et al.*, 1979). The laboratories want to obtain great variety of selected yeast strains for their fermentation characteristics, adapted to the possible styles of wine, with detail to the aromatic and gustative profile, working temperatures, nitrogen needs, influence on colour and alcohol yield, among others (Ribereau-Gayon *et al.*, 2006).

1.6.1. Wine yeast strains

The yeast strains which are involved in winemaking influence fermentation speed, the nature and quantity of secondary products formed during alcoholic fermentation and the aromatic characteristics of the wine (Ribereau-Gayon *et al.*, 2006).

The criteria for the selection of yeast strains are based upon the choice of yeasts being able to improve the quality and consistency of wine (Comi *et al.*, 1997). The selection of yeast strains depends on their oenological characteristics, such as fermentative rate, tolerance to alcohol and to SO_2, flocculent characteristics, the presence of killer factors, acetic acid production, H_2S production, malic acid metabolism, higher alcohol production, alcohol yield, glycerol production, acetaldehyde production and thermic tolerance (Barre, 1980; Darriet *et al.*, 1988; Degre, 1989; Dubourdieu *et al.*, 1988; Duteurtre *et al.*, 1990; Giudici *et al.*, 1993; Valade and Rinville, 1990; Zambonelli *et al.*, 1994).

There is a multitude of yeast strains and the ability to differentiate between the different strains of *S. cerevisiae* is required for the following fields: the ecological study of wild yeasts responsible for the spontaneous fermentation of grape must; the selection of strains with the best oenological qualities; production and marketing controls; the verification of the implantation of selected yeasts used as yeast starter; and the constitution and maintenance of wild or selected yeast collections (Ribereau-Gayon *et al.*, 2006).

Nevertheless, through the last years, wineries are facing new challenges due to current market demands and climate change effects on the wine quality (Teixeira *et al.*, 2013). Different yeast strains (such as *S. cerevisiae* and *S. cerevisiae* var. *cerevisiae*) can contribute to solve some of these challenges.

In French Institute of Vine and Wine (2005, 2011), different oenological characteristics were studied through two different commercial active dried yeast stains, FERMOL® SUPER 16 and ICV D80®, in a synthetic media with a potential alcoholic strength of 12% volume (Table 2).

Table 2 – Oenological characteristics of two different commercial active dried yeast stains, FERMOL® SUPER 16 and ICV D80®, in a synthetic media with a potential alcoholic strength of 12% volume (French Institute of Vine and Wine, 2005, 2011)

Oenological characteristics	**FERMOL® Super 16**	**ICV D80®**
Strain	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae* var. *cerevisiae*
Time of latency	Medium	High
Yeast killer phenotype	Sensible	Killer
Kinetics of fermentation	Fast	Fast
Ratio sugar/ethanol	16.3	16.5
Residuals sugars (g/l)	1	13
Degradation of malic acid (%)	24	25
Production of volatile acid (g/l H_2SO_4)	0.25	0.07
Production of SO_2 (mg/l)	< 20	< 15
Production of acetaldehyde (mg/l)	27	19
Production of pyruvic acid (mg/l)	6	1
Production of glycerol (g/l)	9.8	5.9

In both yeast strains, the kinetics of fermentation, ratio sugar/ethanol and degradation of malic acid had similar results. However, it should be noted that in the residual sugars parameter, the yeast strain ICV D80® shows a higher measure comparing with FERMOL® SUPER 16 yeast strain. Furthermore, ICV D80® shows a yeast killer phenotype characteristic with a killer category and FERMOL® SUPER 16 shows sensitive. Killer yeasts secrete toxic proteins or glycoproteins that inhibit sensitive cells of the same or other species of yeasts (Breinig, 2006). Relatively to the kinetics of fermentation, the tracking of the yeast population can be useful to monitor and control fermentations.

1.6.2. Biotransformation by yeasts

During alcoholic fermentation carried out by yeasts, glucose and fructose are transformed/metabolised into various products, mainly alcohols, and other secondary products like polyols, fat acids, organic acids and many volatile compounds (Moreira, 2015). These molecules, mainly formed from precursors, such as volatile alcohols, esters, acids, terpenes and carbonyl compounds, already present in the grape berry, can undergo transformation and change wine characteristics and complexity perception (Figure 15) (Ebeler and Thorngate 2009; Robinson *et al.*, 2014).

However, the ability of yeasts' derivates to affect wine flavour and aroma is related to four main properties: the ability of yeast walls to bind aroma molecules, their characteristic of flavour enhancers, the ability of yeast macromolecules and colloids to affect the volatility of wine aroma, and the release of volatile compounds into the wine (Lubbers *et al.*, 1994; Dziezak, 1994; Chalier *et al.*, 2007; Pozo-Bayon *et al.*, 2009; Comuzzo *et al.*, 2006, 2011).

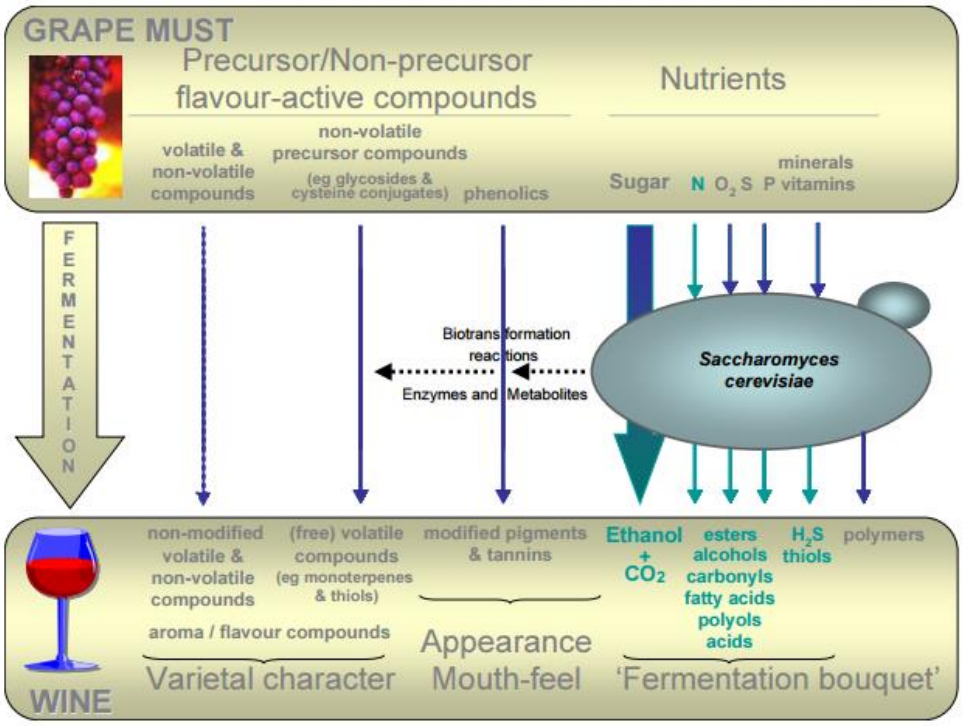


Figure 15 – Main chemical compounds of grape must and wine that suffer biotransformation. Adapted from Bell and Henscheke, 2005

Grape must potential, can be divided, into:

- Precursor or non-precursor flavour-active compounds: volatile and non-volatile compounds, non-volatile precursor compounds and phenolics;
- Nutrients: sugar, nitrogen, oxygen, sulphur, potassium, minerals and vitamins.

Several biotransformation reactions during fermentation occur in grape must with numerous enzymes and metabolites in the juice. So, the wine acquires the following potential:

- Varietal character: non-modified volatile and non-volatile compounds and free volatile compounds;
- Appearance mouth-feel: modified pigments and tannins;
- "Fermentation bouquet": ethanol, CO_2, esters, alcohols, carbonyls, fatty acids, polyols, acids, thiols, polymers.

1.7. Fermentations

1.7.1. Alcoholic Fermentation (AF)

After glycolysis, when oxygen levels are very low, alcoholic fermentation takes place (Goold *et al.*, 2017). Pyruvic acid subsequently may be decarboxylated to acetaldehyde, which is reduced to ethanol by the transfer of electrons from NADH (see Figure 7, topic 1.4.2.1.) (Jackson, 2008).

Wine is usually batch-fermented, so the nutrient availability is maximal at the beginning of fermentation, and declines progressively thereafter (Díaz-Montaño, 2013). By the end of fermentation, most sugars have been metabolized, leaving the wine "dry" (Rodríguez-Porrata *et al.*, 2008). In kinetics fermentation, the batch fermentations generally show a growth pattern consisting of four phases – lag, log, stationary and decline (El-Mansi *et al.*, 2012) (Figure 16).

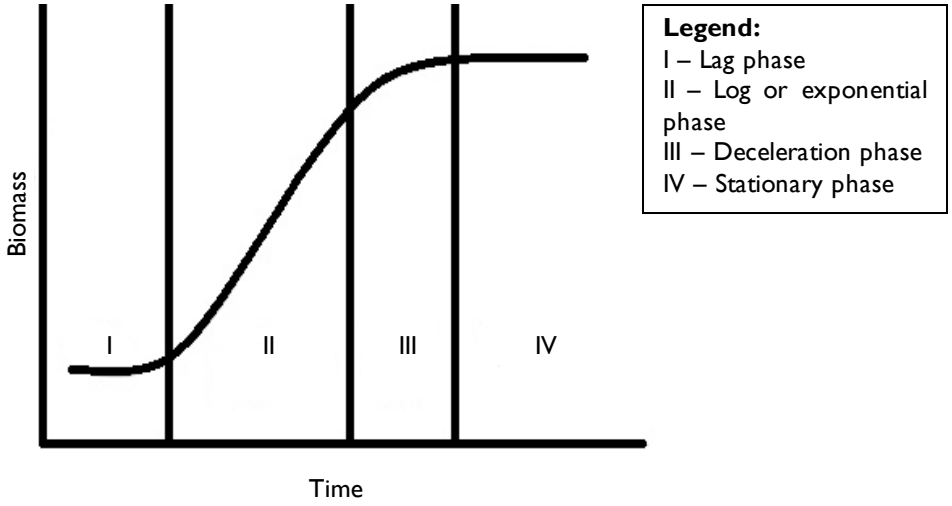


Figure 16 – Kinetics fermentation

Following dry yeast inoculation, cells need to adjust to the new environment and some cells do not acclimate successfully. In this initial period, the number of new cells produced approximates the number that die - lag phase. Once adaptation is complete, most cells begin to multiply at a steady rate, until conditions become unfavourable. As well as most microorganisms are unicellular, the growth curve approximates an exponential equation, and the phase is correspondingly called the exponential or log (logarithmic) phase. During this period, the population of viable cells rapidly increases to its maximum value. As the nutrient content falls, toxic metabolic by-products accumulate. Thus, after a period of rapid growth, the rate of cell division declines and approaches the rate at which cells die or become metabolically inactive - stationary phase. As nutrient conditions continue to deteriorate and the concentration of toxic metabolites keeps increasing, more cells die than divide (Jackson, 2008). According to Jin *et al.* (2012), the culture enters a decline phase at this point, because most viable cells are not replaced, the colony eventually perishes or becomes dormant.

From a winemaking point of view, the lag phase is short or undetectable and may result from the preadapted state of the cells initiating fermentation. The exponential growth and the stationary phase is relatively short and the last phase mentioned may be short and start before nutrients become limiting. As much as 40% of the sugar metabolized to alcohol may occur during the decline phase (Ribéreau-Gayon *et. al.*, 2006).

Excessive temperatures, sugar concentrations, nutritional deficiencies and inhibition phenomena can provoke sluggish or stuck fermentations. So, there are several oenological products that winemakers add to control the fermentation process (Jackson, 2009).

After completing alcoholic fermentation, wine may be treated to foster a second fermentation – the malolactic fermentation. Malolactic fermentation is particularly valuable in cool climatic situations, where a reduction in acidity ameliorates the wine's taste characteristics (Jackson, 2008).

1.7.2. Malolactic fermentation (MLF)

The malolactic reaction takes place at the interior of the lactic acid bacteria, mainly *Oenococcus oeni* (Jackson, 2008). It is a biological process of wine in which the dicarboxylic L-malic acid, or malate, is converted into the monocarboxylic L-lactic acid, or lactate, and carbon dioxide (Figure 17) (Davis *et al.* 1985). *Oenococcus oeni* is the preferred species used to conduct MLF due to its acid tolerance and flavour profile produced (Liu, 2002).

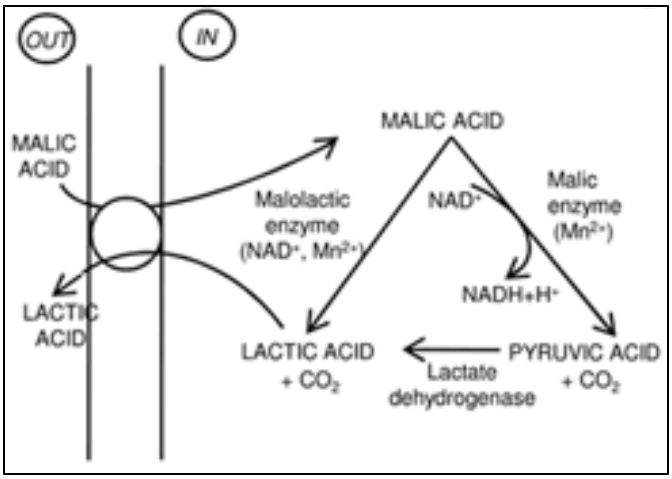


Figure 17 – Malolactic fermentation process (Pessione *et al.*, 2010)

The decarboxylation of malic acid is performed either by means of the malolactic enzyme with Mn^{2+} and NAD^{+} binding domain, that catalyses one step reaction or through the malic enzyme which produces an intermediate compound, pyruvate, which is then reduced to lactate (Denayrolles *et al.*, 1994; Lonvaud-Funel, 1999).

MLF also makes excessively acidic wines more acceptable to the human palate and may improve microbial stability by removing residual fermentable substrates (Jackson, 2008).

1.8. Sensory evaluation

Beyond physico-chemical and microbiological analysis, sensorial analysis is also applicable.
Sensory tests are made in the characterization and evaluation of food and drinks, but also in other fields such as environmental odours, personal hygiene products, diagnosis of illnesses and testing of pure chemicals (Meilgaard *et al.*, 2006).
Tasting wine has always been a part of the standard operations of a winery and its techniques are applicable in quality control, product and market development and research (Stone *et al.*, 2012).
Sensory evaluation is a scientific discipline used to evoke, measure, analyse, and interpret reactions to stimuli perceived through the senses (Stone *et al.*, 2012). Sensory tests are conducted according to protocols minimizing physiological and psychological biases that could affect the sensory response of the sensory panellists (Lawless and Heymann, 1998).
There are several techniques and methods of sensory evaluation, however the preference mapping and triangular techniques are mainly used in wineries.
Preference mapping is a technique that can be used to explore relationships between competitive products in a given category and to group consumers who have similar tasting patterns (Lesschaeve, 2007). This technique has been used to study consumer preferences and when there are many products (Meilgaard *et al.*, 2006).
In the triangular procedure, two samples are known to be alike and the third different and judges would be asked to pick the odd sample from among the three. This test is a scientific method with many uses in sensory evaluation such as the gauging if an overall difference is present between two products and determining whether modifications in processing or ingredients added have significantly changed a product or not (Amerine *et al.*, 1965).

1.9. Food safety and wine

Nowadays, food safety represents an absolute priority and the wine makers should be able to ensure this because wine is vulnerable to hazards and has a potential to cause adverse health effects.
Due to the efficacy of the Hazard Analysis Critical Control Point (HACCP) system to guarantee food safety and, at the same time, product quality, the world wine industry also applies, or should apply, this methodology. Wine, like any other food product, can be

contaminated with substances harmful to health during its production, processing, bottling, storage, transportation and distribution. Therefore, throughout the production chain, strict health standards should be respected and they must be permanently implemented.

In *Enclos des Anges*, the HACCP is not applied, but all hygiene techniques and general hygiene are respected. However, the winemakers of *Enclos des Anges* have knowledge and skills to intercede and put corrective measures in case of hazard situations.

The most efficient way of evaluating wine evolution is through physical-chemical, sensory and microbiological analyses. However, in a traditional cellar wine situation, there are many parameters that winemaker can easily control, such as the temperature, pH and SO_2.

During the whole winemaking process, the temperature should be respected and wine conditions should not be favourable to the spoilage of bacteria and yeast.

The addition of SO_2 is traditionally considered as an efficient method to protect and preserve the wine at different stages of its production. However, this component has a limit dosage for each type of wine, in case of dry red wines with < 5 g/l of sugars is 150 mg/L by the Commission Regulation (EC) n°606/2009. Despite the fundamental reactions outlined above, in high concentrations, sulphur dioxide is well known as a poisonous and allergenic substance (LD_{50}: 0.7-2.5 mg/kg body weight depending on animal species; maximum daily intake: 0.7 mg/kg b.w.), and for this reason it could have a strong impact on the perception of the consumers as regards human health, such as warning "contains sulphites" found on the large majority of wine bottles (Figure 18) (Ribereau-Gayon *et al.*, 2006; Zironi *et al.*, 2006). Pregnant women's exposure to a SO_2 might affect the normal processes that take place in her child as it grows and such adverse effects might include a child with learning deficits or problems in social behaviour (Agency for Toxic Substances and Disease Registry, 1998).

Figure 18 – *Enclos des Anges* red wine label

In order to be commercialized within the European Union (EU), wine intended for human consumption is subject to certain presentation and labelling requirements which aim to protect the interests of consumers and producers.

In wine bottle there are compulsory particulars and optional particulars mentions in label. The compulsory mentions for wine are (Commission Regulation (EC) n° 1308/2013 and n° 607/2009):

- the designation for the category of the grapevine product;
- protected designation of origin or a protected geographical indication;
- the actual alcoholic strength by volume;
- an indication of provenance;
- an indication of the bottler;
- an indication of the importer in the case of imported wines;
- Declaration of the content of allergenic ingredients.

The vintage year is optional, however the net quantity shall be expressed in units of volume in the case of liquid products (Commission Regulation (EC) n°1169/2011).

The *Enclos des Anges* company label follow the European Union laws with criteria of Corsica regulations.

However, before labelling and checking the labels, the wine goes through an ageing process where it is important to be controlled. *Acetobacter* bacteria could develop in it and makes the wine vinegar. Winemakers must take into account the development of this microorganism and prevent it from being a potential hazard.

Chapter 2
Objectives

2. Objectives

The aim of this work was to test the effect of two dried commercial yeast strains, ICV D80® and FERMOL® SUPER 16, in chemical, fermentative process and quality perspective in *Enclos des Anges* Syrah red wine from the same harvest. This study was performed in different stages of winemaking process: before, during and after fermentations.

In the ageing stage, the two wine samples experimented different situations:

- Routine analysis: performed in the department of the food processing chemistry of the Departmental Laboratory of Analyses of Haute-Corse in Bastia on 31st January 2017;
- Reference analysis: performed in the Institute for Higher Education in Vine and Wine Sciences in Montpellier from 27th February till 3rd March 2017.

To accomplish this, two questions are addressed:

- Do the two dried commercial yeast strains produce two different wines, in a chemical and sensorial perspectives?
- Are the two dried commercial yeast strains different in winemaking process?

Chapter 3
Materials and Methods

3. Materials and Methods

3.1. Cultivation conditions and pre-fermentative stage

In *Enclos des Anges* the Syrah field has an area of 3 hectares with 4044 grapevines per hectare (Figure 19).

Figure 19 – Syrah field of *Enclos des Anges* company. Adapted from Google Earth

Gobelet is the grapevine growing technique and this technique accomplishes many objectives, such as the exposure of leaf area to maximize the interception of light, the optimization of the leaf area to fruit ratio and the best disease control (Reynolds and Heuvel, 2009). Furthermore, the grapevines were planted in 2008 (Figure 20), using a R110 rootstock at 2.75m spacing between rows and 0.9m distance between individual vines.

Figure 20 – Syrah grapevine in 2008
Source: Marjorie Spurr

In addition, the Syrah clone 300 grapevines were selected according to its agronomic characteristics and quality wines produced (Table 3). This clone presents moderate levels of blight symptoms and has increased upright growth (INRA-IFV-Montpellier SupAgro, 2017).

Table 3 – Agronomic and quality characteristics of clone 300 Syrah grapevine (INRA-IFV-Montpellier SupAgro, 2017)

	Parameters	Clone 300
Agronomic data	**Fertility**	Medium
	Weight of grape bunches	Low
	Size of berries	Low
	Production level	Medium
	Sensitivity to Botrytis	Medium
Technical data	**Sugar content**	Medium to high
	Potential colour	Medium
	Tannic structure	Medium
	Oenological aptitude	Round wines with good olfactory intensity

Furthermore, relatively to the technical parameter, clone 300 has a medium character in potential colour and tannic structure.

Considering the soil, its composition has about a pH value of 6.9 and the majority oligo-elements are magnesium and iron (see Attachment 1).

Moreover, the winemaker put the organic fertilizer, OvinAlp MV 100, in winter season of 2015 to fertilize the soil and improve the development of grape vines. Additionally, the product with complex microelements, Brexil Duo from Valagro, was put in Syrah field to improve the humus of the soil, to prevent the medium and low deficit and to stimulate the growth leafs. This product was applied in three different times: before anthesis (around January 2016) and after anthesis (in March and in July 2016).

The temperature is another important factor and the average temperature in France and North of Corsica can be compared in Figure 21. As showed the average temperature of North Corsica is lower than in France average temperature.

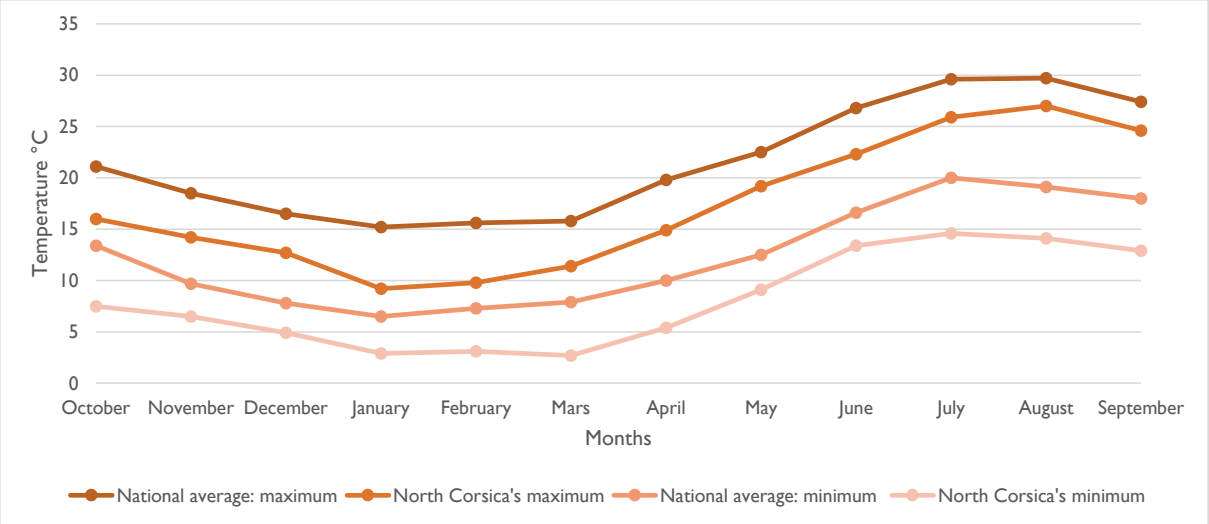


Figure 21 – France temperature average and north Corsica's temperature from October 2015 till September 2016. Adapted from *Linternaute*, 2015 and 2016

The rainfall was not intense in winter, spring and summer seasons in north Corsica comparing with France national average (Figure 22).

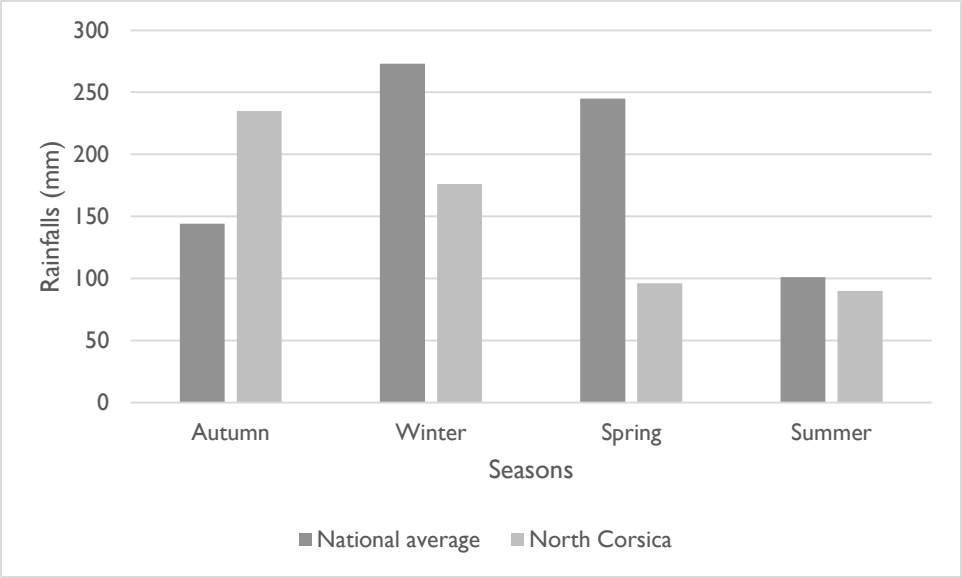


Figure 22 – France rainfalls average and north Corsica's rainfalls from autumn 2015 till summer 2016. Adapted from *Linternaute*, 2015 and 2016

After the maturation process of grape berries the harvest was performed in September 2016. All the harvested product was brought to *Enclos des Anges* company to initiate the winemaking pre-fermentative process, as described in topic 1.5.

3.2. Before fermentation

Before fermentation, several analysis were performed, namely the potential alcohol content (PAC), pH, temperature, and total acid (TA).

Two hundred of Syrah grape berries were collected and measured in balance model from *BALCO Sartorius* (Germany). Then, the must was manually obtained through crushing and the weight of the must and the solid parts of grape berries, like the seeds and skins, was also measured.

The PAC was determinated with hand refractometer, model from ALLA (France) (Figure 23). This equipment is important to track grape maturation and measure the sugar content of grapes (Son *et al.*, 2009).

This refractometer contains two measures, the potential alcohol concentration in percent by volume and apparent density. However, just the PAC value was used in this study, because it is not always possible to obtain a representative grape or must sample for analysis with hand refractometer, due to (Ribereau-Gayon *et al.* 2006):

- these measurements are affected by other substances released into the sample from the grape and other sources;

- the concentrations of these substances are different for every grape or grape must sample;
- the conversion rate of sugar into alcohol varies and depends on fermentation conditions and yeast properties.

This apparatus was calibrated with deionized water at 20°C before using the must sample. After the must sample is placed on the surface, the natural light passes through the sample, entered the measuring prism and falls on the measuring scale where it can be read.

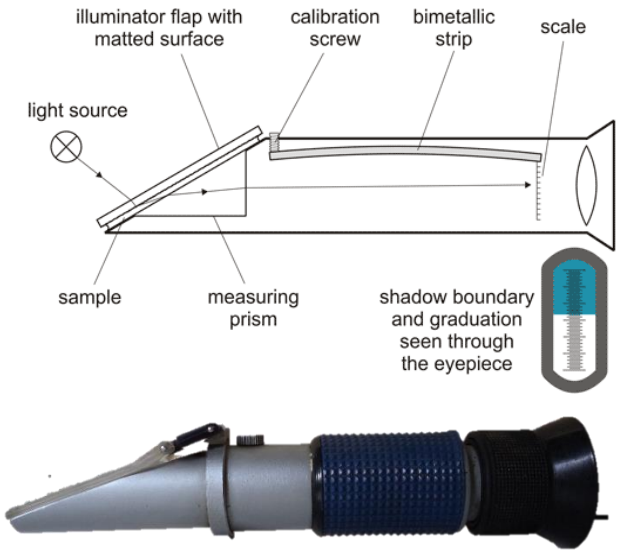


Figure 23 – Hand refractometer with Baume scale.
Adapted from Harris (2005)

Relative to the pH and temperature, these parameters were analysed with the kit pH7 Portable/pH Meter, model from XS Instruments (Italy) (Figure 24).

Figure 24 – pH 7 Portable/pH Meter

The must was put into flask and the temperature and pH were measured with an electrode, model from XS Sensor (Italy), following the instructions of the equipment.

Finally, the total acidity was measured. This was measured by a titration method using a sodium hydroxide (0.1N) solution from *Laboratoire Dujardin-Salleron* (France) as titrant with 1/10mL burette support, model from COREX (USA). Before the titration, three drops of bromophenol blue with 10ml of must were added to the flask.

3.3. During alcoholic fermentation

During this process, different procedures were used, such as:

- Addition of dried yeasts, nutritive complex and di-ammonium phosphate (DAP);
- Oenological techniques: pump-over, devatting, heat and stirring lees.

ICV D80® (*Institute Coopératif du Vin* D80®) and FERMOL® SUPER 16 (SPINDAL SARL) commercial yeast strains were added in each tank *LAINOX* with an amount of approximately 20g/l (see Attachments 6, 7 and 8). The tanks are made of stainless steel material (n. 304) with a cooling jacket and a polished interior surface. The capacity of the studied tanks are 5.200 litres which correspond to 7.5 tons of grape berries (Figure 25).

Figure 25 – Wine tanks and pump-over machine in *Enclos des Anges* company

The nutritive complex added, the NUTRICELL FINISH, contain yeast autolysates with a high amount of amino acids and yeast cell envelopes that are very useful for the treatment of stuck fermentation (see Attachment 3). It has the purpose of reactivating alcoholic fermentation in the event of difficult conditions, such as nutrient deficiency, high clarification and high alcohol levels.

Relatively to DAP added, the ENOVIT® (SPINDAL SARL) is a yeast development activator in the fermentative phase and contains 70% ammonium sulphate, 19.8% diamonnium phosphate, 10% chemical inert filter aid and 0.2% vitamin B1 (see Attachments 4 and 5).

In both oenological products, the quantities added followed the recommended doses (see Attachment 3 and 4).

About oenological techniques, the pump-over, also known as *remontage*, is the process of pumping red wine up from the bottom of the tank and splashing it over the top of the fermenting must. The purpose is to submerge the skins so that CO_2 is pushed to the surface of the must and released. In addition, the devatting process, also known as *délestage*, is the oxidative winemaking process in which, after the cap of grape must, skins, seeds and stems forms on the top of a tank of fermenting wine, the wine is drained through a valve at the base of the tank into another tank and reserved while the remaining solids are allowed to drain for a few hours. The reserved wine is then pumped back into the original tank over the top of the drained skins, seeds and stems. As punch down and pumps over, the purpose of devatting is to increase the extraction of colour, flavour, tannins and aromas from the solids, as well as aerate the fermenting wine.

The pump-over process was used in most of the days during fermentation and the stirring lees were removed in the last pump-over practiced. In the last day of alcoholic fermentation, the electric heater cane was used inside of tanks to extract easily the phenolic compounds.

Furthermore, temperature and volumetric mass in two tanks was monitored daily at the same time. The two tanks have a thermometer *LAINOX* incorporated and the temperature was taken from the screen of the thermometer. Moreover, the volumetric mass was measured using the mustimeter of Dujardin-Salleron in an average temperature of 20°C (Figure 26).

Figure 26 – Mustimeter of Dujardin-Salleron

In addition, winemakers request many routine analyses to the laboratory and they taste the wine frequently to check tannins and aromas and to make sensorial analysis.

3.4. After fermentations

Routine and reference analyses were performed in this study in different phases:

- Routine analyses were performed in the department of the food processing chemistry of the Departmental Laboratory of Analyses of Haute-Corse in Bastia on 31st January 2017;
- Reference analyses were performed in the Institute for Higher Education in Vine and Wine Sciences in Montpellier from 27th February till 3rd March 2017.

The routine and reference methods were performed after three and four months after the beginning of ageing stage, respectively.

3.4.1. Routine analysis methods

All of the routine analyses were performed on a Fourier Transform Infrared Spectroscopy (FTIR), FTIR AVATAR 370, model from Thermo-Nicolet, with *BACCHUS* Acquisition Software and sample display M.S.U. *Cetim* (France).

The spectroscopic, spectrophotometric and separate methods are considered to be the simplest and therefore widely used for carrying out wine samples (Correia, 2011). They are widely used in areas of production and quality control in several industries, such as

pharmaceutical, food, textile or petrochemical industry (Correia, 2011). FTIR allows a rapid and reproducible multi-parameter analysis with little or no need for sample preparation, being a robust, rapid and easy technique (Versari *et al.* 2010; Moreira and Barros, 2002).

The infrared (IR) spectrum is produced by recording changes in absorption of IR radiation by molecules, which undergo mechanical motions, mainly vibrational and rotational modes, due to the absorption of energy (Diem, 1993; Guillen and Cabo, 1997).

FTIR uses interferometric modulation of radiation to measure multiple frequencies simultaneously, producing an interferogram that is recalculated using complex algorithms to give the original spectrum (Figure 27) (Rodriguez-Saona and Allendorf, 2011). The interferometer has an internal reference laser, the frequency of which is known accurately, making it an internal calibration standard of wavelength (Bauer *et. al.*, 2008).

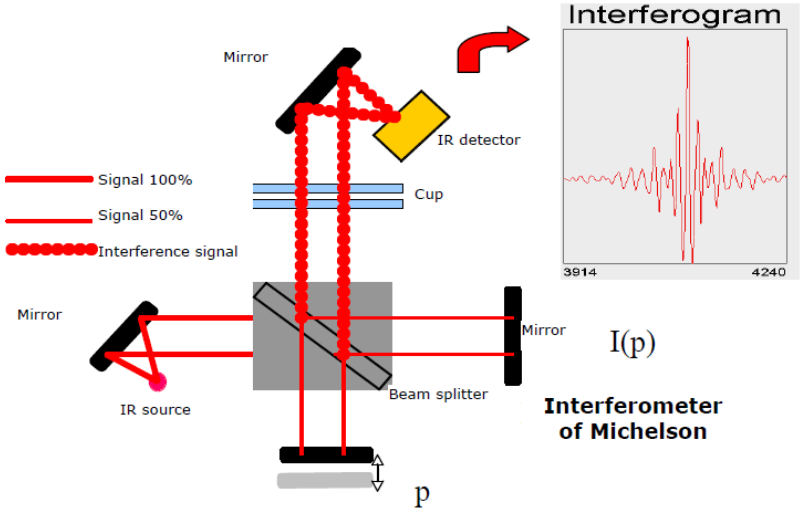


Figure 27 – FTIR spectrometer. Adapted from Frederico, 2010

The Bacchus Acquisition System (Figure 28) was the analyser based on FTIR spectroscopy, allowing the quantification of essential routine wine parameters, such as PAC, reducing sugars, total acid, volatile acid, pH, free SO_2, total SO_2, malic acid and lactic acid. This system can analyse samples and adjust parameters, in function of each sample. Results can be read directly on a screen, which allows the selection and optimization of different calibrations and these are easily manageable. Equipment maintenance, such as washing program, and a robotics test are integrated (Correia, 2011).

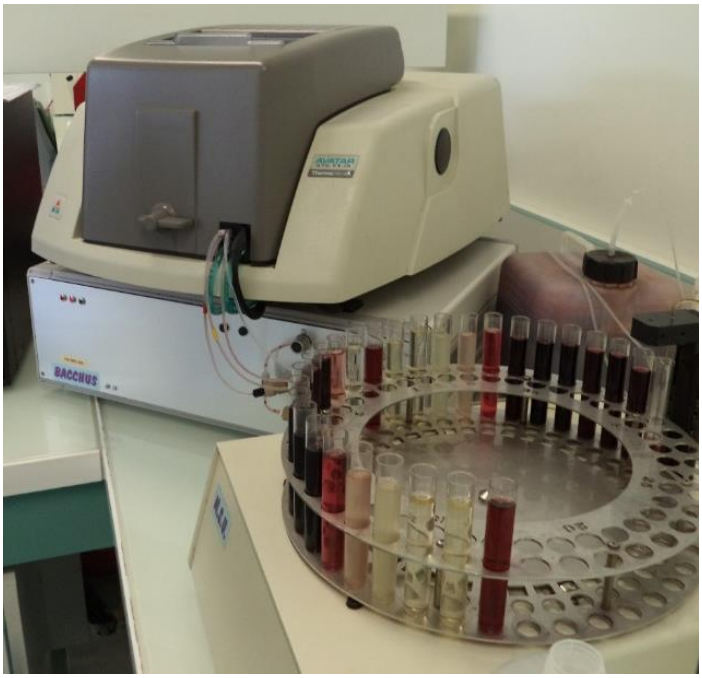


Figure 28 – FTIR equipment and test tubes with wine samples in test tubes support

The ICV D80® and FERMOL® SUPER 16 samples were prepared in test tubes and they were put in test tubes support, after calibration of the instrument for various compounds. These samples can simultaneously titrate the referred compounds from the wine samples.

3.4.2. Reference analysis methods

The reference analyses were performed following the Compendium of International Methods of Analysis of Wines and Musts from International Organisation of Vine and Wine edition 2017, which is a reference of scientific, legal and practical interest. The Compendium's distinguished role in the standardisation of methods of analysis helps to facilitate international trade.

The analyses were performed in the oenological laboratory and all the reagents were prepared by laboratory technician previously. These were executed in triplicate for each parameter.

Lastly, the determination of tartaric, malic and lactic acids were made by chromatography paper analysis following an intern method.

3.4.2.1. Alcohol strength (AS)

The alcoholic strength by volume is the number of liters of ethanol contained in 100 liters of wine, both volumes being measured at a temperature of 20°C. This analyse was performed with the method of obtaining distillate and it is expressed by the symbol % vol (OIV, 2017a).

3.4.2.2. Reducing sugars

Usually, the reducing sugars are detected by as dilution of the sample, in order to decrease the quantity of sugar. In this case, wine is considered dry, so the amount of sugar is low (< 5 g/l) and there is not necessary to dilute the sample.

The quantity of sugar, expressed as invert sugar, contained in the sample is given in the Table 4 as a function of the volume of sodium thiosulfate used, obtained by the method OIV-MA-AS311-01A (OIV, 2017a). This volume was acquired from titration of the sample.

According to OIV (2017a) the sugar content of the wine must be expressed in grams of invert sugar per liter to one decimal place, account being taken of the dilution made during clarification and of the volume of the test sample.

Table 4 – Table giving the relationship between the volume of sodium thiosulfate solution and the quantity of reducing sugar in mg. Adapted from OIV (2017a)

$Na_2S_2O_3$ (ml 0.1M)	Reducing sugars (mg)
1	2.4
2	4.8
3	7.2
4	9.7
5	12.2
6	14.7
7	17.2
8	19.8
9	22.4
10	25.0

3.4.2.3. Total acidity (TA)

According to OIV (2017a) the total acidity of the wine is the sum of its titratable acidities, mainly tartaric, malic and citric acids, when it is titrated to pH 7 against a standard alkaline solution.

During the titre, the bromothymol blue was used as indicator and the titre was completed with an end-point colour standard.

The total acidity is expressed in grams of sulfuric acid (H_2SO_4) per liter and is given by the volume of H_2SO_4 (*A*), though the formula TA = 0.049 x *n*, where *n* is *A* x 10, following method OIV-MA-AS313-01 (OIV, 2017a).

3.4.2.4. Volatile acidity (VA)

This analysis is derived from the acids of the acetic series present in wine in the free state and combined as salts (OIV, 2017a). A small amount of acetic acid results from alcoholic fermentation of sugars (Correia, 2011).

The volatile acidity in grams of sulphuric acid (H_2SO_4) per liter is given by the formula VA = 0.245 (*n* – 0.1 *n*' – 0.05 *n*"), where *n* is the volume of sodium hydroxide and *n'* and *n"* is the volume of iodine. These volumes were obtained in three distinct titrations, following the method OIV-MA-AS313-02 (OIV, 2017a).

3.4.2.5. pH

The volume of pH was obtained with one electrode from pH Fisher Scientific Bioblock (France). After the calibration of the pH apparatus from Inolab WTW (Germany) (Figure 29), the ICV D80® and FERMOL® SUPER 16 wine samples were measured.

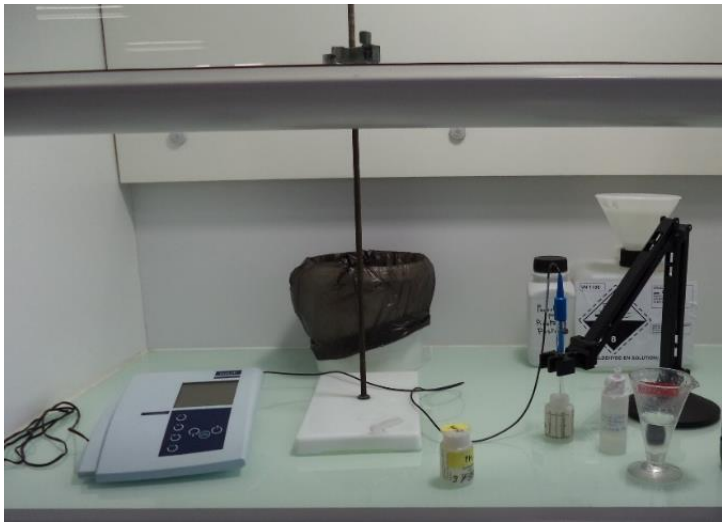


Figure 29 – pH apparatus in hotte with temperature control

3.4.2.6. Free SO_2

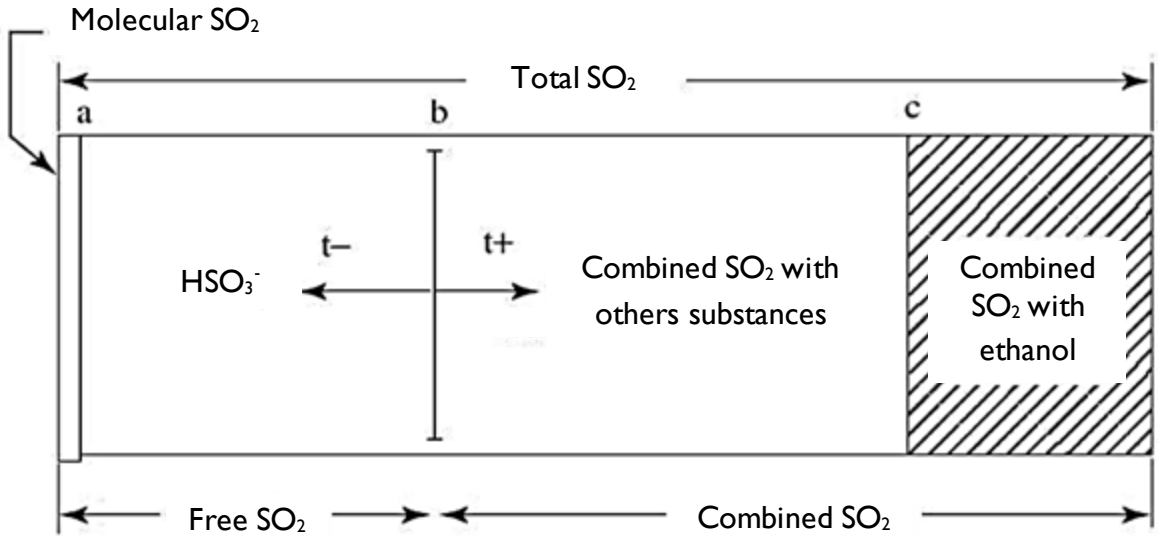
According to OIV (2017b), free sulphur dioxide is defined as the sulphur dioxide present in the must or wine, as its molecular form (SO_2) and ionic form (HSO_3^-) considering the pH and the temperature constant (equation 1). This method was described by Franz Paul (Figure 30), following the method OIV-MA-AS323-04A (OIV, 2017b).

Equation 1: $H_2SO_3 \leftrightarrow H^+ + HSO_3^-$

Figure 30 – SO_2 determination by Franz Paul Method

3.4.2.7. Total SO_2

The total SO_2 is defined as the total of all the various forms of sulphur dioxide present in wine, either in the free state or combined with other constituents (Figure 31) (OIV, 2017b).
The analysis' method of this component is similar of free SO_2, however sulphur dioxide is purged from the wine by entrainment at high temperature, which is approximately 100 °C, following the method OIV-MA-AS323-04A (OIV, 2017b).



Legend: **a** - the boundary between the molecular form and bisulphite; **b** - the boundary between the free and combined fractions with other compounds; **c** - the boundary between the fraction combined with ethanol and the fraction combined with other compounds.

Figure 31 – Interaction between the different components of SO_2.
Adapted from Ribéreau-Gayon *et al.*, 2006

Chapter 4
Results and Discussion

4. Results and Discussion

4.1. Before fermentation

Table 5 represents some monitoring analysis of Syrah grape berry before fermentation in *Enclos des Anges*.

Table 5 – Monitoring analysis of Syrah grape berry in *Enclos des Anges* company

	Weight of 200 grape berries (g)	**Weight of must from 200 grape berries (g)**	**Weight of solid parts from 200 grape berries (g)**	**PAC (%Vol)**	**TA (g/l H_2SO_4)**	**pH**	**Temperature (ºC)**
Syrah grapevine	263.5	76.2	181.9	14.2	3.53	3.70	26.7

The three weight measures, grape berries, must and solid parts, had the aim to prepare the winery, to calculate the number of tanks needed. It can also give information to the commercial company area, mainly to assess whether is possible to produce more rosé wine. This measure indicates how much water the berries have and whether there are ripe or not. Therefore, it is possible to extrapolate the final quantity of wine that can be obtained.
The data of PAC was performed from refractometer and this measure was based on the proportional diffraction of light by the sugar in grape juice (see Figure 23, topic 3.2). Yeasts need 16.8 g/l of sugar to produce 1% alcohol vol. and for Syrah grapevine clone 300R with *Enclos des Anges* viticulture techniques, the PAC was 14.2% vol. This value is estimated because it is based in a small sample, which cannot reflect the entire harvest.
Total acidity was controlled in order to access its evolution before and after fermentations and its value is expected to low after fermentations, according to Silva *et al.* (2003).

This organic acids, detected in TA, affect directly the pH and they are essential mainly for stabilization of colour, because the increase of pH causes the discoloration of anthocyanins (Rosado, 2013). The 3.7 pH value prevents microbial contamination in grape berries and it was in the allowed range of 2.80 and 3.90, according to Cardoso *et al.* (2005).

The temperature influences many chemical reactions and the pH. The value of temperature 26.7°C, which is higher than environmental temperature (≈ 25°C), can enhance fruit maturation, acid metabolism and a rise in pH juice (Jackson, 2008).

4.2. During alcoholic fermentation

The Figure 32 represents the monitoring of alcoholic fermentation following volumetric mass and temperature parameters, for a period of 25 days, in two different yeast studied, the ICV D80® and FERMOL® SUPER 16. The days of addition of ENOVIT® and NUTRICELL FINISH are also represented.

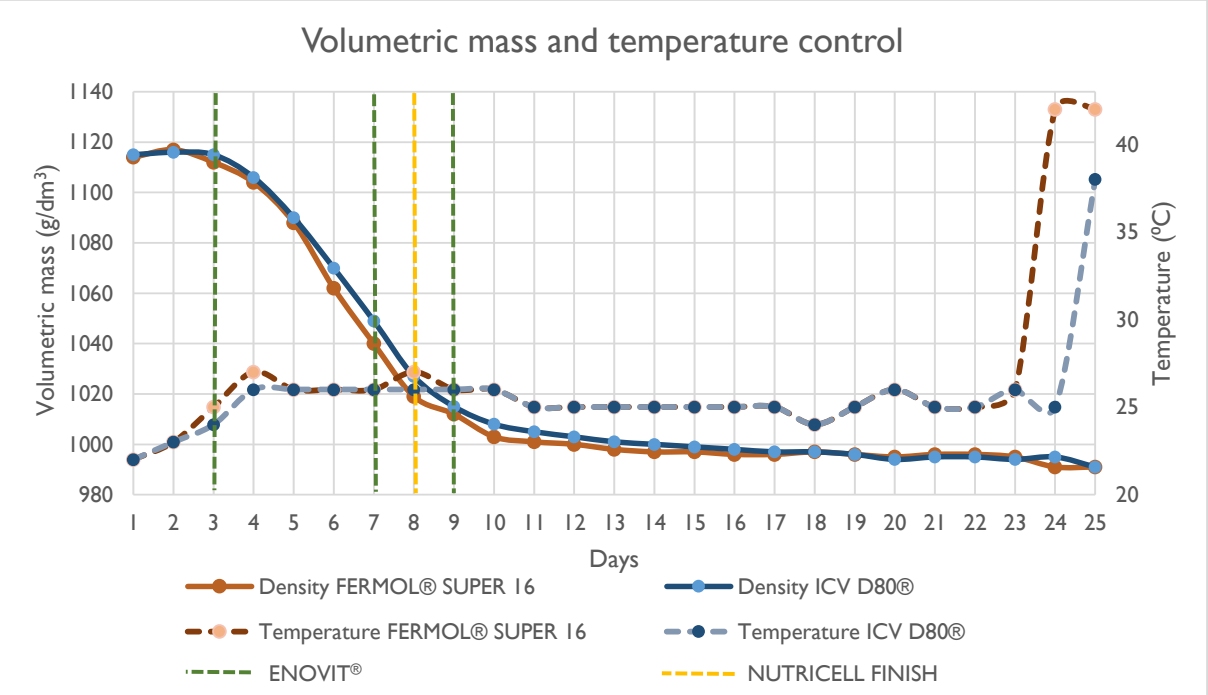


Figure 32 – Monitoring alcoholic fermentation following volumetric mass and temperature parameters, for a period of 25 days, in two different yeast strains, the ICV D80® and FERMOL® SUPER 16. The ENOVIT® and NUTRICELL FINISH products were added in four different times

In both tanks tested, ICV D80® and FERMOL® SUPER 16, the temperature and volumetric mass results obtained were similar (Figure 32). During all AF, various oenological techniques were applied, such as pump-over, devatting and stirring lees. However, these techniques were done more often since the 10th day of AF.

In the first day, the yeasts were put in the two tanks. After this and in the following day, the mixture was homogenized which allowed the inoculation and multiplication of the yeasts. In this phase, there is not a significant consumption of sugar, so the values of volumetric mass remain approximately the same. In third day, the bio-activator nitrogen-vitamin rich in nutritive elements and development growth factors that stimulate the biological activity of yeasts, the ENOVIT® oenological product, was added. It also contains vitamin B1 vitamin that allows a balance in the fermentation, in order to ensure yeast growth and good fermentation kinetics. From that day, the sugar began to be consumed by yeasts in a quick way, according to the study carried out by IFV (see Table 2, topic 1.6.1). This product was also added in the 7th and 9th days of fermentation, with the same purpose above described. The sugar quantity has decreased along days, because the substrate becomes limiting. Both yeast strains had a similar behaviour, however, FERMOL® SUPER 16 yeast strain had a faster sugar consumption than ICV D80® yeast strain. This may correspond to the latency time, because ICV D80® has a latency time higher than FERMOL® SUPER 16.

On the eighth day of fermentation, the NUTRICELL FINISH oenological product was added to resynthesize sugar transport proteins and thus stimulate alcoholic fermentation. This nutritive complex was put into fermenting tanks to make sure that all sugar is fermented and to prevent stuck fermentation in the next stages.

In addition, fermentation continued slowly after the 10th day, which may indicate that there was less sugar substrate to consume, according to the values of volumetric mass that are almost stabilised. This values range between 1003 and 991 for FERMOL® SUPER 16 strain and between 1008 and 991 for ICV D80® strain. The high amount of ethanol molecules present in the mixture can also influence the decrease fermentation rate.

The temperature was not homogeneous in the tanks, so this measure was controlled after pump-over or devatting process, to ensure a more accurate measure, because the must was homogenised.

Till the 4th day, the temperature rised in both tanks due to multiplication of yeasts and an increase fermentation activity which resulted in a release of high quantities of by products, mainly heat.

After this, till the 23rd day, the temperature seemed to be the same along time because sugar levels decrease which can lead to a low fermentation activity and therefore a less release of heat. However, between the 18th and 20th days, it can be detected a small temperature variation in both tanks. It may be explained by environmental conditions or a human error because it occurred in both tanks.

In the final stages of AF, from the 23rd till 25th days, the temperature was increased with heater apparatus following winemaker techniques, reaching around 40°C. This rise of temperature had the aim to extract certain compounds, such as polyphenols from the skins of the wine, which are good for ageing stage.

Comparing both yeast strains, the FERMOL® SUPER 16 showed high values of temperature in first days of AF, which indicates that this strain has a slightly higher fermentation activity than ICV D80®.

Both tanks finished AF with 991 of volumetric mass with high temperature with 38°C for ICV D80® and 42°C for FERMOL® SUPER 16.

4.3. After Fermentations

Table 6 represents the analysis obtained by routine and reference methods of the two Syrah wines with different yeast strains.

Table 6 – Analysis of the two Syrah wines with different yeast strains, ICV D80® and FERMOL® SUPER 16, performed by routine and reference methods.
* reducing sugars value correspond to the ml of 0.1M $Na_2S_2O_3$ from Table 4
** malic and lactic acid analysis were not performed by reference method

		AS (% Vol)	Reducing Sugars (g/l)	TA (g/l H_2SO_4)	VA (g/l H_2SO_4)	pH	Free SO_2 (mg/l)	Total SO_2 (mg/l)	Malic Acid (g/l)	Lactic Acid (g/l)
ICV D80®	Routine	15.50	2.10	3.30	0.40	3.36	18.00	70.00	0.00	1.40
	Reference	15.02±0.12	2.40*	3.30±0.01	0.33±0.03	3.50±0.03	20.05±0.81	72.00±2.12	**	**
FERMOL® SUPER 16	Routine	16.00	2.00	3.31	0.55	3.40	22.00	73.00	0.00	1.50
	Reference	15.72±0.06	2.40*	3.38±0.00	0.61±0.02	3.44±0.02	21.33±1.33	74.93±2.01	**	**

4.3.1. Routine *vs* reference methods

It should be noted that both analysis were executed with different previous conditions. Considering routine analysis, the time between samples were collected and arrived to the departmental laboratory took 2 hours, which did not interfere so much with the sample. Relatively to the reference analysis, there were 36 hours between the collection of the sample and the arrival at the laboratory of IHEV. Moreover, the sample was subject to temperature and pressure variation as it travelled by air, which may interfere slightly with it.

In the two cases, the samples were transported in a wine glass bottle, each one of 75 cl. In reference methods, the malic and lactic acid analysis could not be performed accurately, but the chromatography paper with lactic and malic acid analysis was performed in order to verify the presence of both organic acids.

Data presented on Table 6 of reference methods are the mean and standard deviation of three successive analysis. Therefore, the values of this method are considered more robust and accurate, but it should be taken in account that the time between the collection of the samples and laboratory tests may have influenced them. In routine analysis, performed by FTIR spectrophotometry, the laboratory did not perform the duplicate or triplicate sample unless the laboratory technique found an anomaly or an error in the results.

In both samples tested, all parameters were the result of yeast and bacterial biotransformation, except the two parameters of SO_2 that corresponded mainly to the addition by the winemaker. The values obtained for ICV D80® and FERMOL® SUPER 16, in both methodologies were similar, because each parameter of routine method is in the range or very close of reference methods.

4.3.2. ICV D80® *vs* FERMOL® SUPER 16 yeast strains

The value of alcohol strength after fermentation must be constant along time. Relatively to the AS analysis, the yeast strain FERMOL® SUPER 16 obtained a higher value in both methodologies comparatively to the other ICV D80® strain. According to IFV (2005 and 2011), FERMOL® SUPER 16 strain has a PAC of 17% vol., whereas ICV D80® strain has a PAC of 16% vol., which can explain the values obtained for each strain.

However, the strains studied demonstrated good alcohol production. This may be related to the high concentration of sugars in the Syrah grape berries, as well as high volumetric mass, which led to higher values of ethanol by biotransformation of yeast. This may be related also with the type of grape vine clone (Table 3, topic 3.1), where it is stated that it has medium/high values of sugars content.

After the fermentations, the sugar content is very low and is not sufficient to allow the microbial growth in sufficient quantities to change the alcoholic degree, and the addition of SO_2 after fermentations makes it even more difficult to increase this content. According to OIV (2016a), the actual alcohol content of wine should not be less than 8.5% vol. Nevertheless, taking into account climate, soil, vine variety, special qualitative factors or specific traditions of certain vineyards, the minimum total alcohol content may be reduced to 7.0% vol. by legislation

particular to the region considered (OIV, 2016a). Furthermore, the wine shall have a total alcoholic strength of not more than 15% vol., however the upper limit for the total alcoholic strength may exceed 15% vol. for wines with a protected designation of origin which have been produced without enrichment (Commission Regulation (EC) nº 1308/2013).
Moreover, both yeast strains showed a high quantity of alcohol and the alcohol strength of dry red wine should not be more than 16% vol., being the results obtained acceptable.

Relatively to reducing sugars, which are substances no longer degraded by yeasts, the values were around 2.0 g/l, which is an acceptable low value. In routine method, ICV D80® yeast strain, presented a slightly higher value, 2.10 g/l, comparing to FERMOL® SUPER 16 yeast strain. This agrees with the IFV study (see Table 2, topic 1.6.1), where the ICV D80® presented a higher value of reducing sugars.
In this oenological parameter, the reference method was obtained in correspondence with the ml 0.1M $Na_2S_2O_3$ from Table 4, and it is an approximate result. In both yeast strains and methodologies, the fermentation processes were complete, due to the lack of sugars to be consumed by yeasts, and wines had conditions to proceed the ageing stage. Moreover, this value must be low in order to another microorganisms, such as *Lactobacillus*, do not consume the residual sugars (Gomes, n. d.). It was also unlikely that wine would re-ferment through action of microorganisms in controlled conditions.

The TA content observed was 3.30 g/l H_2SO_4 in routine method and 3.30±0.01g/l H_2SO_4 in reference method in ICV D80® yeast strain. In FERMOL® SUPER 16 yeast strain the value obtained was 3.31 g/l H_2SO_4 in routine method and 3.38±0.00 g/l H_2SO_4 in reference method. It was observed a slightly higher value of TA in FERMOL® SUPER 16 yeast strain in both methodologies, but the results were very approximate with each other. This reveals that neither of both yeasts have a significant impact in this parameter.

It was observed a higher value of VA in FERMOL® SUPER 16 yeast strain in both methodologies comparing ICV D80® yeast strain. Relatively to the VA oenological characteristic, it was observed 0.40 g/l H_2SO_4 in routine method and 0.33±0.03 g/l H_2SO_4 in reference method in ICV D80® yeast strain. In FERMOL® SUPER 16 yeast strain was observed 0.55 g/l H_2SO_4 in routine method and 0.61±0.02 g/l H_2SO_4 in reference method. According to the IFV study (Table 2, topic 1.6.1), the ICV D80® represents a value of 0.07 g/l H_2SO_4 of VA and the FERMOL® SUPER 16 represents a value of 0.25 g/l H_2SO_4 of VA, which correlates with the

results obtained. It can be said that the FERMOL® SUPER 16 strain wine tank had a higher content of acetic acid comparing with the other study tank, and consequently, a higher probability to develop acetic acid bacteria and produce vinegar wine.
In routine analysis, the VA parameter is essential to notice the final stage of MLF and the ageing stage, because the winemaker can verify if there are episodes of actions of acetic acid bacteria (see Figure 14, topic 1.6). When wine has access to oxygen, acetic acid bacteria can grow in the wine, because these bacteria have alcohol tolerance (Jackson, 2008). According to Commission Regulation (EC) n°606/2009, this parameter for dry wine must not be higher than 0.98 g/lH_2SO_4.

The results of the pH from reference method in both yeast strains were higher comparatively with routine method. However, these values were almost similar considering both yeasts and methodologies. According to Cardoso *et al.* (2005), wines have values of pH between 2.80 and 3.90 and the results obtained belonged to referred interval.

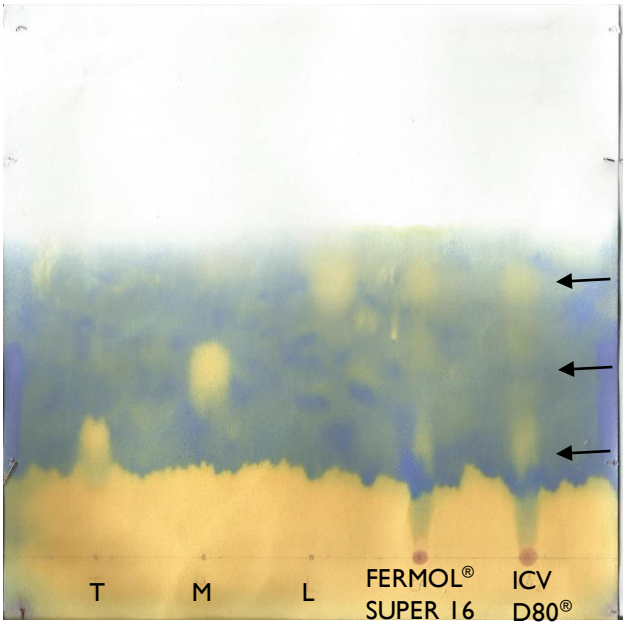
Considering the free SO_2 oenological characteristic, it was possible to observe a lower value in ICV D80® in both methodologies, comparing to FERMOL® SUPER 16 strain. It meant that less molecular SO_2 and bisulphite (HSO_3^-) were present in the sample. Therefore, the ICV D80® probably have a lower antiseptic effect than FERMOL® SUPER 16 yeast strain.

The results of total SO_2 observed were 70.00 g/l in routine method and 72.00±2.12 g/l in reference method in ICV D80® yeast strain. In FERMOL® SUPER 16 yeast strain the results observed were 73.00 g/l in routine method and 74.93±2.01 g/l in reference method. The total SO_2 were higher in FERMOL® SUPER 16 yeast strain than ICV D80®, however, the results were very similar. These are in conformity with law, which have a limit of 150 mg/l in red wines (Commission Regulation (EC) n°606/2009). It proves that the winemaker put a correct quantity of SO_2 after fermentation stages.

In malic acid analysis, the content showed 0.00 g/l which meant that the FML was completely finished because there was no malic acid left to be transformed into lactic acid. Observing lactic acid in the routine method, 1.40 g/l were observed in ICV D80® and 1.50 g/l in FERMOL® SUPER 16 strains. Regarding malolactic fermentation, routine analysis are important to the winemaker to know when the FML is finished.

Lactic acid bacteria have alcohol tolerance and they may grow in favourable conditions, such as, high temperature and pH (Gomes, n. d.). However, the pH of the all samples was less than 3.50 and is unlikely that lactic acid bacteria develop after FML.

In Montpellier IHEV, in order to access the qualitative amount of the tartaric, malic and lactic acids analysis was performed by chromatography paper (Figure 33).



Legend:
T – Tartaric acid;
M – Malic acid;
L – Lactic acid;
ICV D80® – ICV D80® wine sample;
FERMOL® SUPER 16 – FERMOL® SUPER 16 wine sample.

Figure 33 – Tartaric, malic and lactic acid using chromatography analyse paper

The chromatography analysis paper showed the three acids on the left side and the two samples on the right side. In both wine samples, it was observed the distribution of two correspondent acids, mainly tartaric acid and lactic acid. It correlates with the results previously discussed.

4.4. Sensory evaluation

According to winemakers of *Enclos des Anges* company, both wines produced very full bodied highly aromatic wines the typical ripe berry flavours of blackcurrant, plumps and dark cherries mixed in with black pepper and herbal spice notes. The wine with ICV D80® yeast strain may have produced a slightly rounder mouth feel with black olives and perhaps slightly more spicy, whereas the FERMOL® SUPER 16 wine seemed to produce a more sharper mouth feel with a

more floral, like violets, and chocolaty retroflection. In their opinion both wines were very similar in style and it was difficult to differentiate the two easily.

Chapter 5
Conclusion

5. Conclusion

The process of producing a quality wine is complex. In addition, there are many factors inherent to wine production, from soil and climatic factors, namely the choice of the wine growing techniques, to the selection of vine varieties and phytosanitary treatments. Methods and means used during winemaking and microorganism strains also decisively influence the character and style of wine. It can be affirmed that wine is obtained from the interactions between all these factors.

In winemaking process, all stages contribute to the final product and also influence the characteristics of the wine. Thus, knowledge of the winemaking process and all intervenient compounds are essential for effective control. The winemaker is a key factor in the production of a wine in many situations, such as control, taste and interpretation of analytical results.

There are many organisms involved in the producing of wine, for instance, the *Saccharomyces cerevisiae* yeast, which was subject of this study. Answering the first question purposed, the two yeast strains compared, ICV D80® and FERMOL® SUPER 16, do not produce different wines, at the phase of the ageing stage studied. In a chemical perspective, both wines had the same fermentative conditions and the same quantities of oenological products added. Regarding the analyses executed, namely the routine and reference analyses, the quantitative values obtained were very similar. In a sensorial point of view, they produce analogous wines with few tasting differences, according to winemakers' experience. In spite of this, yeast strains are biologically different, which could have resulted in distinct wines.

Relatively to the second question, yeast strains compared are different during winemaking process. FERMOL® SUPER 16 may have demonstrated a higher efficiency in the majority of the parameters observed, namely volumetric mass and temperature during alcoholic fermentation, and alcohol strength, volatile acidity and free and total SO_2 after fermentations. Therefore, this yeast strain can proportionate better profitability during the winemaking process. In spite of this, both yeast strains had the same behaviour in a general outlook.

Each wine cellar adapts its' winemaking process according to their possibilities in accordance with the European laws and specific local regulations.

In all stages of the winemaking process and for all the employees, the hygiene rules are applied to guarantee the conformity of the wines.

Chapter 6
Final considerations

6. Final considerations

6.1. Future perspectives

Wine is one of the most consumed drinks in the world which motivates the winegrowers and winemakers to produce different types of wine to satisfy the consumer. In addition, wine contains various bioactive compounds that are beneficial to consumers' health, so it is important to ensure both situations.

According to the obtained results in this work, more studies should be performed, namely the manipulation of certain conditions to understand their effect in the behaviour of yeast strains in fermentative process. For instance, it could be interesting to test the accurate influence of temperature, sulphur dioxide addition and inoculum levels of yeast strains, ENOVIT® and NUTRICELL FINISH. Additionally, the organic fertilizer and other viticulture products in the vintage may be manipulated to test the impact of them in the wine.

However, the oenological techniques during fermentations should be studied in greater depth, mainly the application of pump-over and devatting processes more frequently per day.

Also the HACCP can be implemented in *Enclos des Anges* company to analyse the hazards and define the control points and the critical control points associated with the winemaking process.

Another issue that could be discussed is the application of genetically modified organisms to favour each wine region, but it is still a controversy situation.

6.2. Personal reflexion

All stages and processes, from the vintage till wine bottling, were very challenging for me. I had the opportunity to be abroad with ERASMUS programme in Corsica Island and France mainland during this study. I met many realities, such as cellars, laboratories, investigations, wine tasting, wine fairs and oenology clubs.

It brought me an opportunity to gain more skills and to understand the different winemaking realities. However, this is an area that have to be more explored because there is many different techniques, products and oenological processes around the world. In Portugal the products and techniques used in winemaking process may be different from Corsica because there are many factors, mainly the natural environment, agronomic factors and culture, which

influence the wine procedures. Additionally, Portugal has many quality wine and vine regions and it is important to focus on trainees and investigations, namely in food safety and quality control, because the consumers are more demanding. The frauds and adulterations in wines starts to be present and it is a health, ethic, dietary, sensory, ecological concern.

The Food Safety Master at Faculty of Pharmacy in University of Coimbra should develop more the wine theme, because the production of wine is as important as the production of another food product.

Chapter 7
Bibliography

7. Bibliography

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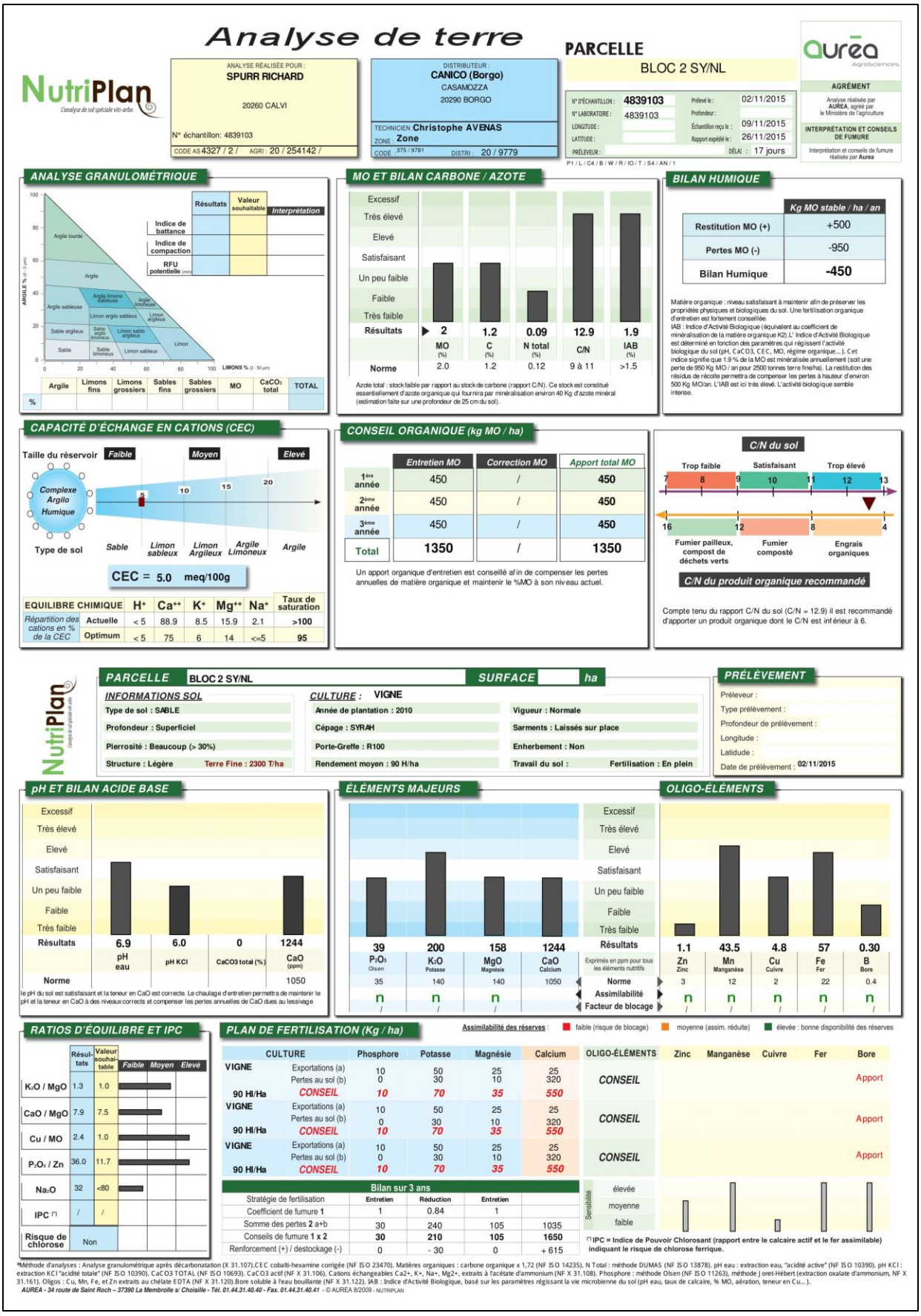
Chapter 8
Attachments

Attachment 1 – Analysis of the Syrah grapevine field



Analyse de terre

NutriPlan — L'analyse de sol spécialisé viti-arbo

Aurea AgroSciences — AGRÉMENT — Analyse réalisée par AUREA, agréé par le Ministère de l'agriculture — INTERPRÉTATION ET CONSEILS DE FUMURE — Interprétation et conseils de fumure réalisés par Aurea

ANALYSE RÉALISÉE POUR : SPURR RICHARD
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N° LABORATOIRE :	4839103	Profondeur :	
LONGITUDE :		Échantillon reçu le :	09/11/2015
LATITUDE :		Rapport expédié le :	26/11/2015
PRÉLEVEUR :		DÉLAI :	17 jours

P1 / L / C4 / B / W / R / IO / T / S4 / AN / 1

ANALYSE GRANULOMÉTRIQUE

	Résultats	Valeur souhaitable	Interprétation
Indice de battance			
Indice de compaction			
RFU potentielle (mm)			

	Argile	Limons fins	Limons grossiers	Sables fins	Sables grossiers	MO	$CaCO_3$ total	TOTAL
%								

MO ET BILAN CARBONE / AZOTE

	MO (%)	C (%)	N total (%)	C/N	IAB (%)
Excessif					
Très élevé					
Elevé					
Satisfaisant					
Un peu faible					
Faible					
Très faible					
Résultats	2	1.2	0.09	12.9	1.9
Norme	2.0	1.2	0.12	9 à 11	>1.5

Azote total : stock faible par rapport au stock de carbone (rapport C/N). Ce stock est constitué essentiellement d'azote organique qui fournira par minéralisation environ 40 Kg d'azote minéral (estimation faite sur une profondeur de 25 cm du sol).

BILAN HUMIQUE

	Kg MO stable / ha / an
Restitution MO (+)	+500
Pertes MO (-)	-950
Bilan Humique	-450

Matière organique : niveau satisfaisant à maintenir afin de préserver les propriétés physiques et biologiques du sol. Une fertilisation organique d'entretien est fortement conseillée.
IAB : Indice d'Activité Biologique (équivalent au coefficient de minéralisation de la matière organique K2). L' Indice d'Activité Biologique est déterminé en fonction des paramètres qui régissent l'activité biologique du sol (pH, $CaCO_3$, CEC, MO, régime organique...). Cet indice signifie que 1.9 % de la MO est minéralisée annuellement (soit une perte de 950 Kg MO / an pour 2500 tonnes terre fine/ha). La restitution des résidus de récolte permettra de compenser les pertes à hauteur d'environ 500 Kg MO/an. L'IAB est ici très élevé. L'activité biologique semble intense.

CAPACITÉ D'ÉCHANGE EN CATIONS (CEC)

CEC = 5.0 meq/100g

EQUILIBRE CHIMIQUE		H+	Ca++	K+	Mg++	Na+	Taux de saturation
Répartition des cations en % de la CEC	Actuelle	< 5	88.9	8.5	15.9	2.1	>100
	Optimum	< 5	75	6	14	<=5	95

CONSEIL ORGANIQUE (kg MO / ha)

	Entretien MO	Correction MO	Apport total MO
1ère année	450	/	450
2ème année	450	/	450
3ème année	450	/	450
Total	1350	/	1350

Un apport organique d'entretien est conseillé afin de compenser les pertes annuelles de matière organique et maintenir le %MO à son niveau actuel.

C/N du sol

C/N du produit organique recommandé

Compte tenu du rapport C/N du sol (C/N = 12.9) il est recommandé d'apporter un produit organique dont le C/N est inférieur à 6.

NutriPlan

PARCELLE BLOC 2 SY/NL — SURFACE ha

INFORMATIONS SOL

- Type de sol : SABLE
- Profondeur : Superficiel
- Pierrosité : Beaucoup (> 30%)
- Structure : Légère — Terre Fine : 2300 T/ha

CULTURE : VIGNE

- Année de plantation : 2010
- Cépage : SYRAH
- Porte-Greffe : R100
- Rendement moyen : 90 H/ha
- Vigueur : Normale
- Sarments : Laissés sur place
- Enherbement : Non
- Travail du sol : Fertilisation : En plein

PRÉLÈVEMENT

- Préleveur :
- Type prélèvement :
- Profondeur de prélèvement :
- Longitude :
- Latidude :
- Date de prélèvement : 02/11/2015

pH ET BILAN ACIDE BASE

	pH eau	pH KCl	$CaCO_3$ total (%)	CaO (ppm)
Excessif				
Très élevé				
Elevé				
Satisfaisant				
Un peu faible				
Faible				
Très faible				
Résultats	6.9	6.0	0	1244
Norme				1050

le pH du sol est satisfaisant et la teneur en CaO est correcte. Le chaulage d'entretien permettra de maintenir le pH et la teneur en CaO à des niveaux corrects et compenser les pertes annuelles de CaO dues au lessivage

ÉLÉMENTS MAJEURS

	P_2O_5 Olsen	K_2O Potasse	MgO Magnésie	CaO Calcium
Excessif				
Très élevé				
Elevé				
Satisfaisant				
Un peu faible				
Faible				
Très faible				
Résultats	39	200	158	1244
Exprimés en ppm pour tous les éléments nutritifs				
Norme	35	140	140	1050
Assimilabilité	n	n	n	n
Facteur de blocage	/	/	/	/

OLIGO-ÉLÉMENTS

	Zn Zinc	Mn Manganèse	Cu Cuivre	Fe Fer	B Bore
Excessif					
Très élevé					
Elevé					
Satisfaisant					
Un peu faible					
Faible					
Très faible					
Résultats	1.1	43.5	4.8	57	0.30
Norme	3	12	2	22	0.4
Assimilabilité	n	n	n	n	n
Facteur de blocage	/	/	/	/	/

RATIOS D'ÉQUILIBRE ET IPC

	Résultats	Valeur souhaitable	Faible	Moyen	Elevé
K_2O / MgO	1.3	1.0			
CaO / MgO	7.9	7.5			
Cu / MO	2.4	1.0			
P_2O_5 / Zn	36.0	11.7			
Na_2O	32	<80			
IPC (*)	/	/			
Risque de chlorose	Non				

PLAN DE FERTILISATION (Kg / ha)

Assimilabilité des réserves : faible (risque de blocage) — moyenne (assim. réduite) — élevée : bonne disponibilité des réserves

CULTURE		Phosphore	Potasse	Magnésie	Calcium
VIGNE	Exportations (a)	10	50	25	25
	Pertes au sol (b)	0	30	10	320
90 Hl/Ha	CONSEIL	10	70	35	550
VIGNE	Exportations (a)	10	50	25	25
	Pertes au sol (b)	0	30	10	320
90 Hl/Ha	CONSEIL	10	70	35	550
VIGNE	Exportations (a)	10	50	25	25
	Pertes au sol (b)	0	30	10	320
90 Hl/Ha	CONSEIL	10	70	35	550

OLIGO-ÉLÉMENTS	Zinc	Manganèse	Cuivre	Fer	Bore
CONSEIL					Apport
CONSEIL					Apport
CONSEIL					Apport

Bilan sur 3 ans				
Stratégie de fertilisation	Entretien	Réduction	Entretien	
Coefficient de fumure 1	1	0.84	1	
Somme des pertes 2 a+b	30	240	105	1035
Conseils de fumure 1 x 2	30	210	105	1650
Renforcement (+) / destockage (-)	0	- 30	0	+ 615

Sensibilité : élevée / moyenne / faible

(*) IPC = Indice de Pouvoir Chlorosant (rapport entre le calcaire actif et le fer assimilable) indiquant le risque de chlorose ferrique.

*Méthode d'analyses : Analyse granulométrique après décarbonatation (X 31.107).CEC cobalti-hexamine corrigée (NF ISO 23470). Matières organiques : carbone organique x 1,72 (NF ISO 14235). N Total : méthode DUMAS (NF ISO 13878). pH eau : extraction eau, "acidité active" (NF ISO 10390). pH KCl : extraction KCl "acidité totale" (NF ISO 10390). $CaCO_3$ TOTAL (NF ISO 10693). $CaCO_3$ actif (NF X 31.106). Cations échangeables $Ca2+$, $K+$, $Na+$, $Mg2+$, extraits à l'acétate d'ammonium (NF X 31.108). Phosphore : méthode Olsen (NF ISO 11263), méthode Joret-Hébert (extraction oxalate d'ammonium, NF X 31.161). Oligos : Cu, Mn, Fe, et Zn extraits au chélate EDTA (NF X 31.120).Bore soluble à l'eau bouillante (NF X 31.122). IAB : Indice d'Activité Biologique, basé sur les paramètres régissant la vie microbienne du sol (pH eau, taux de calcaire, % MO, aération, teneur en Cu...).

AUREA - 34 route de Saint Roch – 37390 La Membrolle s/ Choisille - Tél. 01.44.31.40.40 - Fax. 01.44.31.40.41

Attachment 2 – Routine laboratory analysis results

DEPARTEMENT DE LA HAUTE CORSE

Direction Générale des Services

Pôle d'Actions Territoriales

Direction de l'Environnement
Laboratoire Départemental d'Analyses 2B

REPUBLIQUE FRANCAISE

Bastia, le: 31/01/2017

M. SPURR RICHARD
Cave viticole de la Signoria
Route de l'aéroport
20260 CALVI

RAPPORT D'ESSAIS N° 170100310

Dossier n° VSPURR-170100310-264	170131-03307	170131-03308			
Identification des échantillons	Vin	Vin			
Date de réception:	31/01/2017	31/01/2017			
Heure de réception:	10:43	10:43			
Date de prélèvement:	31/01/2017	31/01/2017			
Prélevé par:	Technicien Chambre d'Agric	Technicien Chambre d'Agric			
Couleur du vin:	Rouge	Rouge			
Millesime:					
Numéro de cuve:	ICV D80	FS16			

Résultats des analyses : Oenologie

Paramètres	*Unités*	170131-03307	170131-03308			
Titre Alcoométrique Volumiq	% Vol	**15.5**	**16.0**			
Sucres réducteurs	g/l	**2.1**	**2.0**			
Acidité totale	g/l H_2SO_4	**3.30**	**3.31**			
Acidité volatile	g/l H_2SO_4	**0.40**	**0.55**			
pH	Unite pH	**3.36**	**3.40**			
SO2 libre	mg/l	**18.0**	**22.0**			
SO2 total	mg/l	**70.0**	**73.0**			
Acide malique	g/l	**0.0**	**0.0**			
Acide lactique	g/l	**1.4**	**1.5**			

Le Directeur, Pharmacien, Biologiste
Dr Marc MEMMI

Le Responsable de Chimie Agro-Alimentaire
Franck GELORMINI

TOUT COURRIER DOIT ETRE ADRESSE IMPERSONNELLEMENT A MONSIEUR LE PRESIDENT DU CONSEIL DEPARTEMENTAL
LDA HAUTE-CORSE - Parc Technologique Erbajolo - 20600 BASTIA
Tel : 04.95.59.50.50 - Fax : 04.95.59.50.59

Attachment 3 – NUTRICELL FINISH technical sheet

MARTIN VIALATTE

NUTRIENTS

NUTRICELL FINISH

Complex nutrient,
for perfect control of the final stage of alcoholic fermentation

CHARACTERISTICS

NUTRICELL FINISH is a complex nutrient containing all the ingredients needed to:

- ensure that the final stage of alcoholic fermentation (AF) by yeasts proceeds smoothly
- reactivate stuck fermentation by using a fermentation starter.

OENOLOGICAL PROPERTIES

- **NUTRICELL FINISH** contains a high proportion of yeast autolysates that are especially rich in amino acids. When added halfway through AF, these amino acids are assimilated by active dry yeasts (ADY) to resynthesize sugar transport proteins and thus reactivate AF. The autolysates also release vitamins and trace elements needed for proper metabolism of yeasts.
- **NUTRICELL FINISH** also contains yeast cell envelopes (yeast hulls), which due to their detoxifying effect improve fermentation in the event of yeast stress (low temperature, high clarification, high alcohol levels, etc). The hulls are especially useful for the treatment of stuck fermentation.

APPLICATIONS

- Added during AF to reactivate alcoholic fermentation of red, white and rosé wines in the event of difficult conditions (nutrient deficiency, high clarification, high alcohol levels, etc).
- In the event of stuck fermentation, added to wine containing residual sugars before adding a second fermentation starter.

DOSAGE

Recommended dose: 20 to 40 g/hL.
Maximum legal dose according to current European regulations: 160 g/hL.

INSTRUCTIONS FOR USE

Dissolve **NUTRICELL FINISH** in 10 times its weight of water or must.
Add to the batch to be treated. Mix thoroughly.
Precautions for use:
Product for oenological and specifically professional use.
Use in accordance with current regulations.
75/2016 – 1/2

SAS SOFRALAB - 79, av. A.A. Thévenet - CS 11031 - 51530 MAGENTA - France
Tél. : + 33 3 26 51 29 30 - Fax : + 33 3 26 51 87 60 - **www.martinvialatte.com**

SOFRALAB

MARTIN VIALATTE

NUTRIENTS

PACKAGING

1 kg and 10 kg bags.

STORAGE

Store unopened, sealed packaging away from light in a dry, odour-free environment.
Once opened use rapidly.
Use before best-by date stamped on packaging.

75/2016 – 2/2

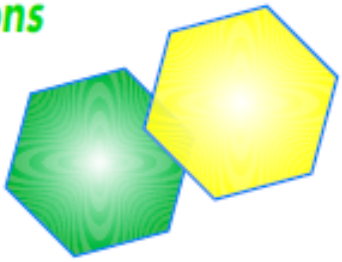
SAS SOFRALAB - 79, av. A.A. Thévenet - CS 11031 - 51530 MAGENTA - France
Tél. : + 33 3 26 51 29 30 - Fax : + 33 3 26 51 87 60 - **www.martinvialatte.com**

SOFRALAB

Attachment 4 – ENOVIT® technical sheet

Activateur des fermentations et refermentations

Enovit ®



ENOVIT®

ENOVIT® EN CAVE

FERMENTATION A BASSES OU HAUTES TEMPERATURES

est un bio-activateur azote-vitaminique riche en éléments nutritifs et facteurs de croissance qui stimule l'activité biologique des levures. L'emploi d'ENOVIT® se justifie dans différents domaines:

Début de fermentation et fermentation en blanc. ENOVIT® permet une multiplication rapide des levures sous leur forme la plus active et facilite ainsi le démarrage des fermentations.
Dans les moûts blancs appauvris en levures par le pressurage et le débourbage, ENOVIT® accélère le démarrage de la fermentation.
Prévention des arrêts de fermentation: ENOVIT® par apport de vitamine B1, il rétablit l'équilibre des moûts carencés assimilables: moûts issus de raisins provenant de terrains pauvres, moûts de vendanges trop mûres ou atteints de pourriture nécessitant un sulfitage important.
Le renouvèlement constant de la flore levurienne est assuré par l'apport des éléments de croissance consommés, compte tenu du fait qu'après 36 heures de fermentation, les levures épuisent les réserves nutritives avec risque de ralentissement ou même d'arrêt de la fermentation.

ENOVIT® permet donc d'obtenir une meilleure résistance de la flore levurienne aux conditions difficiles de la fermentation alcoolique et par conséquent une meilleure transformation des sucres du moût.
Grâce à la vitamine B1 contenue dans ENOVIT®, la production d'acides cétoniques (acide piruvique, acide cétoglutarique) lors de la fermentation alcoolique est faible.
Les vins ainsi obtenus sont plus résistants à l'oxydation et présente un équilibre SO_2 libre SO_2 combiné plus stable.

L'emploi d'ENOVIT® est conseillé dans ce type de fermentation ou en cas d'arrêts de fermentation qui peuvent compromettre gravement le processus fermentaire spécialement dans le cas de vinification en continu.

DOSES D'EMPLOI

15-20 g/hL ou par quintal de produit.

MODE D'EMPLOI

Délayer la dose nécessaire d'ENOVIT® dans de l'eau ou du liquide à fermenter et l'introduire dans la masse lors d'un remontage ou l'asperger uniformément sur les endroits solides par couches de 20 à 30 cm.
Procéder à une aération selon le cas.
Conseils d'utilisation: 10 g/hL de ENOVIT® apporte 20 mg/L d'ARA (azote rapidement assimilable).

NB: dans le cas de milieux pauvres en flore levurienne (moûts débourbés ou centrifugés, marcs, et d'une manière générale tous produits susceptibles de ne pouvoir assurer par eux-mêmes un processus fermentaire régulier et total), l'emploi de levures sélectionnées ***ZYMASIL*** *ou* ***ZYMASIL BAYANUS*** *est conseillé.*

CONDITIONNEMENT

Sachets Doypack de 1kg

Code 000440.

nov 2004

SPINDAL Sarl
ZONE INDUSTRIELLE - 3 RUE AMPERE - 77220 GRETZ-ARMAINVILLIERS
Tél. +33 (0) -1-64078000 - Fax +33 (0) -1-64075920
e-mail : spindal@spindal.fr

Attachment 5 – ENOVIT® safety data sheet

Enovit®

Activateur de croissance des levures en phase fermentaire

Société productrice **Spindal** – Z.I. – 3 Rue Ampère – 77220 Gretz-Armainvilliers – France
Tél : + 33.(0) 1.64.07.80.00 – Fax : + 33 (0) 1.64.07.59.20

Doses d'emploi 30 g/hL ou par quintal de produit
Conditionnement Paquet de 1 kg net en carton de 20 kg. Cod. prod. 000440
Sac de 25 kg net. Cod. prod. 000449

FICHE DE DONNEES DE SECURITE

2 COMPOSITION ET INFORMATIONS SUR LES INGREDIENTS
Composition : E517 Sulfate d'ammonium: CAS 7783-20-2 } Concentration combinée à 89,80%
E442 Phosphate d'ammonium: CAS 7783-28-0 }
E375 Chlorhydrate de thiamine (Vitamine B1): CAS 67-03-8. Concentration à 0,2%
Coadjuvant de filtration chimiquement inerte.

Informations : L'utilisation combinée du Sulfate et Phosphate d'ammonium est légalement limitée à 0,3 g/L.
L'utilisation du Chlorhydrate de thiamine est légalement limitée à 0,6 mg/L.
L'utilisation de l'*Enovit*® est légalement limitée à 30g/hL.

3 IDENTIFICATION DES DANGERS
Eviter de respirer les poussières. Le contact prolongé avec la peau peut provoquer de légères irritations.

4 SOINS D'URGENCE
En cas de contact avec la peau: laver abondamment avec de l'eau et du savon. En cas d'irritation consulter un médecin.
En cas de contact avec les yeux: laver abondamment avec de l'eau pendant au moins pendant 15 minutes. En cas d'irritation consulter un médecin.
En cas d'ingestion: rincer soigneusement la bouche et la gorge avec de l'eau. En cas d'irritation consulter un médecin.
En cas d'inhalation: éloigner le sujet du lieu de l'explosion.

5 MESURE D'INCENDIE
Moyens d'extinctions adéquats : eau, mousse.
Moyens d'extinctions interdits: aucun.
Dangers particuliers: aucun.
Protection contre le feu et les explosions: ne requiert aucune protection particulière.

6 MESURE EN CAS DE FUITES ACCIDENTELLES
Boucher les fuites, récupérer le produit et éviter qu'il finisse à l'égout..
En cas de mise à l'égout, prévenir les autorités compétentes

7 MANIPULATION ET STOCKAGE
Manipulation: éviter la formation de poussière.
Stockage: conserver en lieu frais et sec.
Assurer une bonne ventilation des locaux.

8 CONTROLE DE L'EXPOSITION ET PROTECTION INDIVIDUELLE
Equipement de protection individuelle: masque anti-poussière et lunettes de sécurité.

9 PROPRIETES PHYSICO-CHIMIQUES

Aspect		:	poudre mixte ou granulée de couleur blanche
pH sol. 1%		:	7,8
Poids spécifique	avec tassement	:	1,22
Poids spécifique	sans tassement	:	0,99
ARA (azote rapidement assimilable)		:	10 g/hL d'Enovit® apporte 20 mg/L d'ARA
Thiamine % :		:	0,2

10 STABILITE ET REACTIVITE
Stable aux conditions normales d'utilisation.

11 INFORMATIONS TOXICOLOGIQUES
L'inhalation répétée de poussières peut provoquer des irritations.
Le contact prolongé avec la peau peut provoquer de légères irritations.

12 INFORMATIONS ECOLOGIQUES
Les données sur la mobilité, la persistance, la biodégradabilité, le potentiel de bioaccumulation, la toxicité aquatique et plus en général sur son écotoxicité rendent ce produit non dangereux pour l'environnement.

13 EPURATION
Opérer selon la législation en vigueur.

15 INFORMATIONS SUR LE TRANSPORT
Normes ADR: le produit n'est pas classé

16 INFORMATIONS COMPLEMENTAIRES
Il est bon de rappeler périodiquement aux utilisateurs les risques encourus en utilisant le produit.
La présente fiche technique et de sécurité a été rédigée par les services techniques de la société PASCAL BIOTECH avec le maximum d'informations à sa disposition à la date de la dernière mise à jour. Les informations contenues dans cette fiche font référence et ne peuvent être valables que dans le cas où le produit serait utilisé seul, en cas de combinaison avec d'autres produits ou dans d'autres conditions d'utilisation que celles décrites, rien du contenu de la présente fiche technique et de sécurité ne peut être garanti. Rien du contenu de la présente fiche ne doit être interprété comme une garantie implicite ou explicite. Dans tous les cas, l'application des consignes de sécurité et l'adaptabilité du produit à des usages particuliers propres à l'utilisateur sont sous la responsabilité de ce dernier.
Pour toutes informations complémentaires, écrire ou téléphoner à la société **SPINDAL** - Z.I. – 3 Rue Ampère 77220 Gretz-Armainvilliers – TEL:+ 33 (0) 1.64.07.80.00 – FAX:+33 (0) 1.64.07.59.20
E-mail : spindal@spindal.fr

DATE DE LA DERNIERE MISE A JOUR : 2 décembre 2010

Réalisée en conformité à l'Ann. II du Règlement (CE) n. 1907/2006, relative à l'enregistrement, l'évaluation, l'autorisation et la restriction des substances chimiques. (REACH)

SPINDAL

AEB group

SPINDAL - AEB GROUP – Z.I. – 3 Rue Ampère - 77220 Gretz-Armainvilliers – France
Tél : + 33 (0).1.64.07.80.00 - Fax : + 33. (0).1.64.07.59.20 – E-mail : spindal@spindal.fr- www.aeb-group.com

Attachment 6 – ICV D80® yeast technical sheet

ICV

Siège social :
Institut Coopératif du Vin
La Jasse de Maurin
34 970 LATTES
Tél : 04 67 07 04 90
Fax : 04 67 07 04 95
www.icv.fr

LALVIN ICV D80® SELECTION ICV

Depuis 1979, l'ICV a développé une gamme de levures œnologiques pour les principales applications des caves de vinification.
Comme les autres levures de la gamme ICV, **ICV-D80** est produite, séchée et emballée par Lallemand,
ICV-D80 est une levure naturelle *Saccharomyces cerevisiae* ; elle n'a pas fait l'objet de manipulation génétique lors de son isolement, de sa sélection ou de sa production. Ce n'est pas un organisme génétiquement modifié (non OGM).
ICV-D80 est conforme au Codex œnologique édité par l'OIV et aux normes alimentaires de la FAO, en particulier pour l'absence de métaux lourds et de toxines fongiques.

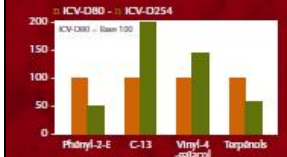
Les certificats de conformité sont disponibles auprès de votre Centre Œnologique ICV ou sur demande écrite à l'adresse ci-dessus : par lettre, fax ou e-mail.

Edition mai 2005

Effet de la levure ICV-D80 sur le profil aromatique et gustatif des vins rouges, Syrah 1994

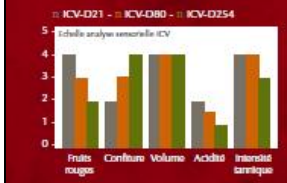
Delteil, Revue des œnologues de Bourgogne, n° 81, 1996

Effet de la levure ICV-D80 sur la concentration en composés volatils des vins rouges, Syrah 1996



Légende :
Phényl-2-E = phényl-2-éthanol : alcool supérieur à odeur de rose
C13 = composé norisoprénoïque en C13 : odeur fruité mûre
Vinyl-4-gaïacol : phénol volatil à odeur épicée et fumée
Terpénols : odeurs florales et fruitées (agrumes).

Complémentarité d'ICV-D80 avec d'autres levures de la gamme ICV. Essai R&D, 2003.



LALVIN ICV D80® SELECTION ICV

Groupe ICV

Levure *Saccharomyces cerevisiae* isolée en 1992 à Ampuis (Côte Rôtie) par l'ICV, dotée du facteur killer (phénotype K2). Cette levure a été sélectionnée pour développer les caractères aromatiques et gustatifs concentrés et puissants des vins rouges méditerranéens. Le choix s'est fait parmi 178 levures différentes de la Vallée du Rhône.

Principales caractéristiques techniques

- Bonne résistance aux conditions difficiles de raisins pour la fermentation alcoolique : faible concentration en azote assimilable, fort titre alcoométrique potentiel. C'est l'une des plus performantes de sa catégorie (achèvement des sucres, AV).
- Forte consommation de SO_2 pendant fermentation, facilitant l'enclenchement de la fermentation malolactique.
- Style aromatique et gustatif original sur les vins rouges issus de longue macération. Acidité plus élevée que sur les vins fermentés avec ICV D47 et ICV D254.
- Couleur vive et stable, intensité tannique élevée avec un grain de tanin très fin et une bonne persistance des arômes floraux, réglissés et fumés.

Précautions d'emploi

- Respect des 13 points clés de maîtrise de la fermentation alcoolique.
- Sensible aux températures élevées (> 28°C)
- Nécessite de nombreuses aérations pendant la phase active de la fermentation, en particulier sur les jus carencés en nutriments et très riches en sucres.
- Susceptible d'amplifier les caractères de maturité tannique imparfaite.

Les utilisations actuelles

- Pleine expression de l'originalité des qualités tanniques dans les macérations longues
- Les vins rouges méditerranéens de haut de gamme pour développer des arômes concentrés et complexes, avec des notes minérales et florales (réglisse, violette), et exprimer de la puissance en bouche avec des tanins à grain très fin et une bonne persistance aromatique finale. Très bonne complémentarité en assemblage avec les vins élaborés avec ICV-D254 et ICV-D21.
- A l'étranger : sur les vins rouges de haut de gamme en Californie et au Chili (Syrah, Merlot, Cabernet).

Attachment 7 – ICV D80® yeast data sheet

GROUPE ICV

Fiche de spécification

www.icv.fr

Version août 2014

Levures Sèches Actives LALVIN ICV

- ICV K1M®
- ICV GRE®
- ICV D47®
- ICV OPALE®
- ICV OKAY®
- ICV D254®
- ICV D80®
- ICV D21®
- ICV THERMOPREMIUM®
- ICV CHARD®

Application

Fermentation alcoolique

Caractéristiques

Ingrédients	Levure sèche active *Saccharomyces cerevisiae*, E491.
Aspect physique	Granulés ronds ou en forme de vermicelles
Couleur	Beige à brun léger
Odeur	Odeur typique de levure fermentaire
Solubilité	Facilement soluble
Producteur	Lallemand
Lieux de production	EU, Canada

NB : la couleur peut varier d'un batch de production à l'autre. Elle ne reflète en aucun cas l'activité fermentaire.
La fiche de données sécurité est disponible sur demande auprès de votre centre œnologique ou sur www.icv.fr

Organisme de production (non OGM)

Saccharomyces cerevisiae
Produit par séchage d'un concentrat de culture levurienne.

Caractérisation produit (en conformité avec le Codex Œnologique)

Cellules viables	> 10^{10} UFC/g
Matière sèche	> 92 %
Coliformes	<10^{2} UFC/g
Escherichia Coli	Absence dans 1 g
Staphylococcus aureus	Absence dans 1 g
Salmonella	Absence dans 25 g
Bactéries lactiques	<10^{5} UFC/g
Bactéries acétiques	<10^{4} UFC/g
Moisissures	<10^{3} UFC/g
Levures de différentes espèces	<10^{5} UFC/g

Métaux lourds (en conformité avec le Codex Œnologique)

Plomb	< 2 mg / kg
Mercure	< 1 mg / kg
Arsenic	< 3 mg / kg
Cadmium	< 1 mg / kg

Conditionnement et conditions de stockage

Emballé sous vide et sous film aluminisé alimentaire en
- Carton outre de 10 kg

ou
- Carton de 10 kg contenant 20 sachets de 500 g

Stocker dans un endroit frais et sec
L'emballage doit être gardé intact. Ne pas utiliser si le paquet n'est plus sous vide avant ouverture.

DLUO : Elle est indiquée sur l'emballage.
Les DLUO au conditionnement sont de 4 ans. Suivre les recommandations et utiliser le produit avant la date limite d'utilisation optimale (DLUO) afin de ne pas devoir augmenter la dose préconisée.
La performance du produit sera optimale si les recommandations de stockage, de dosage, de mise en œuvre et la DLUO sont respectées.

Dosage

Dose recommandée : 20 à 40 g / qt ou hL.

Mise en œuvre

- Toujours réhydrater la levure dans un contenant propre.
- Réhydrater la levure dans 10 fois son poids d'eau tiède (température entre 35°et 40°C).
- Agiter doucement puis attendre 10 à 15 minutes. Agiter doucement une deuxième fois puis attendre 10 à 15 minutes.
- Agiter doucement une dernière fois avant d'ajouter la préparation dans la cuve de fermentation.
  La différence de température entre le mout ou le raisin à inoculer et la suspension de levures ne doit pas excéder 10°C. Si c'est le cas, procéder à une acclimatation progressive (en cas de doute consulter votre œnologue ICV).
- Le temps total de **réhydratation** ne doit pas dépasser 45 minutes.

Les informations ci-dessus sont basées sur les connaissances actuelles disponibles. Elles sont supposées correctes à la date de rédaction. Toutefois la précision et l'exhaustivité des informations sont sans garanties. L'utilisateur est responsable de ses choix de produits, de leurs conditions d'utilisation et des éventuels risques associés.

Attachment 8 – FERMOL® SUPER 16 technical sheet

Fermol® Super 16

LSA spécifique pour la vinification des vins à haut degré d'alcool.

Souche sélectionnée de levure *Saccharomyces cerevisiae* r.ph. *cerevisiae PB 3084 - (INRA - CLIB 2032)*

Sélectionnée par le Laboratoire de Microbiologie Générale - Faculté des Sciences - Université de Reims-Champagne/Ardenne (France).

Souche spécifique sélectionnée pour son activité fermentaire dans des conditions difficiles de températures et de teneur en alcool (34°C et 16-17 % vol), elle est particulièrement indiquée pour la production de vins rouges même à des températures non contrôlées où elle produit peu d'acidité volatile.
Elle permet également d'obtenir d'excellents résultats sur des moûts riches en sucre comme ceux issus de vendanges tardives. Les vins obtenus sont concentrés, les arômes puissants et sucrés rappellant ceux des fruits mûrs.
C'est une souche qui s'adapte parfaitement au milieu, la cinétique fermentaire est régulière, et la production de métabolites soufrés faibles.

Composition et caractéristiques physico-chimiques

- Levure naturelle neutre au facteur killer
- Période de latence courte : < 7H00
- Pouvoir moussant négligeable
- Production d'H_2S négligeable
- Pouvoir alcoogène: 17,4 % vol.
- Rendement sucres/alcool = 17,2 g de sucres/litre pour produire 1% d'alcool vol.
- Production de glycérol: 6,3 à 7,2 g/L.

Produit conforme, présente toutes les qualités requises par la réglementation CE en vigueur.

Stockage

Pour une meilleure conservation et une activité optimale, stocker le produit entre 5 à 7°C.
Une fois l'emballage ouvert, utiliser de préférence dans les plus brefs délais possibles.

Dosage

De 10 à 30 g/quintal de vendange ou hL de moût.

Mode d'emploi

Réhydrater la levure sèche active dans environ 10 volumes d'eau tiède avec du sucre ou des MCR stériles, pendant au moins 20-30 minutes. Introduire ensuite la levure réhydratée en phase de multiplication avancée dans le moût.

Caractéristiques

- Levures vivantes > 2.10^{10}/g.
- Perte de vitalité 10-15% en moyenne par an, en fonction de la température de conservation.
  La date de production est indiquée sur l'emballage.
- Matière sèche 96 ± 1%.

Pour usage œnologique.
Conforme aux normes CE.
Produit non issu d'OGM.

Conditionnement

Paquet laminé de 500 g dans une boîte par carton de 20 paquets.

Code produit : 005318

31622

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