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Study of the technical procedures for the reduction of the ethanol content in wines

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Riassunto

Questa ricerca affronta le conseguenze del riscaldamento globale sul cambiamento climatico, in particolare per quanto riguarda il settore del vino e l'aumento del contenuto di zucchero con un conseguente aumento del titolo alcolometrico. A questo proposito, verranno analizzati gli aspetti negativi e positivi della riduzione dell'etanolo in un vino, considerando varie procedure. Queste varie possibilità per diminuire la gradazione alcolica sono fondamentalmente: strategie viticole, strategie non microbiologiche (come il processo tecnologico basato sulle membrane, la nanofiltrazione, il cono di filatura) e processi microbiologici (per esempio le strategie di ingegneria metabolica, l'uso di lieviti non convenzionali o i nuovi fonti di carbonio in *Saccharomyces cerevisiae* per abbassare il livello di etanolo nel vino). Al giorno d'oggi, l'esigenza di diminuire il contenuto di alcol riguarda anche la salute umana e la preferenza dei consumatori perché i media riportano continuamente le preoccupazioni sull'abuso di alcol e gli effetti sulla società; mentre i benefici per la salute precedentemente associati al consumo moderato di vino sono messi in ombra da prove convincenti e probabili che esiste una relazione causale tra il consumo di alcol e alcuni tumori.

Abstract

This research addresses the consequences of global warming on climate change, particularly regarding the wine sector and the increase in sugar content with a subsequent rise in alcoholic strength. In this respect, the negative and positive aspects of reducing ethanol in a wine will be analysed, considering various procedures. These various possibilities to decrease the alcoholic strength are basically: viticultural strategies, non-microbiological strategies (such as membrane-based technologies process, nanofiltration, spinning cone), and microbiological processes (for instance the metabolic engineering strategies, use of non-conventional yeasts, or the novel carbon sinks in *Saccharomyces cerevisiae* to lowering wine ethanol level). Nowadays, the exigency to decrease the alcohol content also relates to human health and consumer preference because the media continually report concerns about alcohol abuse and societal effects; while the health benefits previously associated with moderate wine consumption are overshadowed by convincing and probable evidence that a causal relationship between alcohol consumption and some cancers exists.

1. Introduction

In recent decades, the mass media have been used by scientists, politicians, and civil society to open their eyes to an increasingly worrying reality, climate change.

For a long time, this problem was ignored, its existence denied, and serious discussions were postponed indefinitely to find concrete solutions to counteract the irreversible advance of what has turned out to be perhaps the greatest universal natural disaster since the beginning of industrialization. Climate change is partly caused and exacerbated by man's actions, by the reckless exploitation of resources, by the inadequate countermeasures adopted to accompany industrial and economic development, and by the selfishness that has driven the human being to want to obtain and accumulate more and more, leading him to forget the morally and socially acceptable and desirable limits; those limits necessary to avoid finding himself at the point of no return, the point that will irreparably damage the Earth.

Nowadays in a globalized world in the 21st century, everybody knows that the consumption of fossil fuels is causing an increase in the concentration of carbon dioxide and other gases, which, by reflecting the radiation back off to earth, are causing a greenhouse effect (Crowley, 2000; Zamora, 2005a) that is responsible for the current global warming of the planet. All agri-environmental sectors, such as viticulture and oenology will have to address major challenges since many biological changes due to global warming will have to be faced, and the increase of the alcohol level in wine-related to a high sugar content is one of them.

The issue is particularly important in this sector, as reducing the alcohol content of wine while avoiding or minimizing quality deterioration and preserving its specificity is now essential for the entire wine sector worldwide. In addition, a high wine alcohol content has negative effects on human health and, presently is not appreciated by a wide part of consumers that prefer drinking light and responsible, e.g., the possible contribution of higher concentrations of ethanol and the intake of ethanol-derived calories to overweight will lead to a worrying consequence for human health, obesity, thus it discourages wine consumption from consumers.

Furthermore, as far as the wine trade is concerned, higher alcohol levels may directly and significantly reduce competitiveness in markets where taxes and/or duties are directly linked to alcohol content.

All of these are motivations that lead us to the main objective of this study, which is how to reduce the alcohol content in wine, or even earlier in the vineyard how to reduce the sugar content in grapes.

But what are the consequences of climate change for grapes/wine?

2. Consequences

Global warming leads to a faster accumulation of sugars and faster degradation of acids in grapes compared to normal conditions (Jones et al., 2005; Mira de Orduña, 2010).
This environmental disaster causes an increasing imbalance between the vine's primary metabolism (in which sugar content, acids and pH are affected) and secondary metabolism (molecules with low molecular weight). This forces grape growers to harvest the grapes before reaching the correct skin and seed maturity, which affects seriously the wine composition and quality.
As we all know, one of the fundamental parameters for good grape quality is precisely phenolic maturity. Particularly in red wine, whose development of the skin and seeds must be optimal to have a good sensory response in the finished product is crucial, as the phenolic maturity in question refers to the extractability of polyphenols and anthocyanins.

Some of the most important climate change-related effects are:

- Advanced harvest time
- Increases in temperatures,
- Increased grape sugar concentration led to the high alcohol content
- Low pH
- Higher titratable acidity

Reports from Johannisberg (Rheingau, Germany), for example, show that the first day of harvest takes place on average 2-3 weeks earlier than it did at the end of the 18th century and the beginning of the 20th century. (Stock, Gerstengarbe, Kartschall, & Werner, 2005). Figure 1 in fact shows very simply how industrial maturity is moving towards not fully ripening the grapes, to avoid harvesting grapes that are far too ripe and with all the consequences seen before (Zamora, 2014).

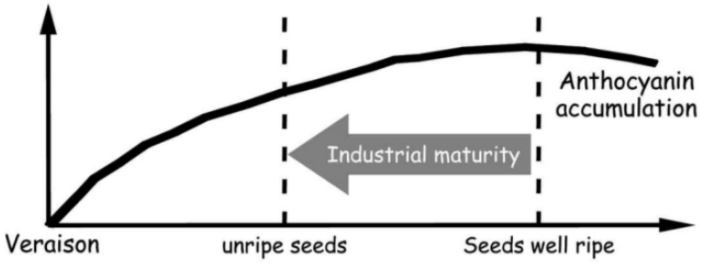

Figure 1 Effects of global warming on grape maturity (Zamora, 2014).

2.1 Impact on Viticulture

2.1.1 Temperatures effects

As we have seen before it is well known that elevated temperatures during berry ripening induce faster pulp maturation and enhance must total soluble solids and pH. Warmer temperatures extend the period during which the minimal temperatures needed for the physiological activity of wine are reached, and hence, augment metabolic rates and affect metabolite accumulation. This is completely true since, for temperatures above 25 °C, photosynthesis decreases even at constant sun exposure (Huglin & Scheider, 1998), and above 30 °C, berry size and weight are reduced, and metabolic processes and sugar accumulation completely stop (Hale & Buttrose, 1974).

However, it seems that temperature effects on final sugar accumulation are reported to be relatively small (Coombe, 1987), but levels higher than 24-25 Brix are not due to photosynthesis and sugar transport from leaves and wood, but to concentration by evaporative loss (Keller, 2009, 2010).

Nevertheless, this change of temperature affects an important wine parameter, acidity. While tartaric acid is relatively stable, malic acid levels are strictly dependent on temperature and decrease with higher temperatures. Usually, a lower acidity is correlated with a high pH, also because high temperatures lead to increased potassium levels which are also temperature-dependent (Coombre, 1987; Hale, 1981). Besides these parameters, other compounds are affected by higher temperatures, in particular phenolic compounds suitable for the sensory characteristic of wine. Their role as photo protectants explains their dependency on sun exposure. However, temperatures have been shown to play a direct and important role in their formation, too. For instance, anthocyanin synthesis is temperature affected, indeed temperatures of 30 °C led to lower anthocyanin production (Buttrose et al., 1971; Spayd et al., 2002; Tarara et al., 2008). Specifically high temperatures directly after veraison can inhibit anthocyanin formation (Mori et al., 2007).

Grape skin and seed-derived proanthocyanidins are important for red wine astringency. Several studies have shown a positive association between temperature and the number of seeds or total proanthocyanidins levels per berry at harvest (del Rio & Kennedy, 2006; Ewart & Kliewer, 1977).

Regarding practical experience on white wine, aromas suggested that aromas develop more favourably in cool climates, thus higher temperatures led to a potentially reducing aromatic intensity (Duchêne & Schneider, 2005).

It is thought that global warming affects vine phenology, vegetative cycles, and grape quality (Jackson & Lombard, 1993; Winkler, Cook, Kliewer, & Lider, 1974), since in the last 30 years we have seen those dates for bud break, flowering and fruit maturity are now earlier in various regions, leading to an advance of harvest dates. Some research even though much longer than reaching back

hundreds of years confirms these trends, e.g., information from Johannisberg (Rheingau, Germany) shows that the first day of harvest now takes place of 2-3 weeks early than it was between the 18th century and the 20th century. In Alsace has been shown that between 1945 and 2000 the average temperature increased by 1.8°C, especially during the ripening phase. A further example was in coastal California areas where the average annual temperature increased by 1.13 °C and the star of growing season advanced 18-24 days between 1951 and 1997 (Nemani et al. 2001).

2.1.2 Carbon dioxide effect

Carbon dioxide is very important for many factors as photosynthesis and growth of the plants. CO_2 has increased since industrialization and research confirm that. Free Air CO_2 Enrichment (FACE) structures were used to study the effect of enriched CO_2 concentrations on vine phenology and physiology under realistic conditions for a variety of studies involving crops, trees, and other plants. (Bindi, Fibbi, Lanini & Maglietta, 2001). Studies conducted by Bindi, Fibbi, Gozzini, Orlandi, Seghi (1997), and Maglietta too (2001) with 20 years-old Sangiovese grapevines found that atmospheric CO_2 levels elevated from current values of $350 - 550\ \mu mol\ mol^{-1}$ increased biomass by 40-50 % as total biomass and dry fruit weight. However, at maturity, no difference could be found among the treatments, and it was concluded that the effect of higher CO_2 concentrations on grape and wine quality were limited and the yield increasing effects may be reduced by the effect of warmer temperatures.

However, more studies involving *Vitis vinifera* in FACE systems will be required to clarify the possible effects of CO_2 variables.

2.1.3 Secondary effects of climate change

In parallel with these primary effects, there are some indirect effects such as increased grape and wine salinity is a phenomenon associated with several semi-arid and arid regions, such as Australia and Argentina. This effect derived attributes, such as “brackish”, “seawater like”, “soapy” that are considered negatively and are correlated with a high concentration of Na, K, and Cl (Walker et al., 2003).

Another effect is that climate change has favoured increased incidence of forest and bushfires (Overpeck, Rind, & Goldberg, 1990). The consequence of these phenomena will undoubtedly be the loss of valuable ecological spaces, but also in areas affected by these phenomena, where smoke has struck the territory (such as Australia and California), the wines are described with the attributes dirty, burnt, or ash provoked by compounds such as guaiacol, 4-methylguaiacol, 4-ethyl-guaiacol, 4-ethylphenol, eugenol, and furfural (Howell, 2008, 2009; Simos, 2008; Vallesi & Howell, 2007);

However, it should be noted that red wines can have a significant guaiacol component due to toasted oak barrels.

One more climate change-related factor is the prominence of various pests and diseases, as well as the vectors responsible for disease distribution. One important example is Pierce's disease caused by *Xylella fastidiosa* (the bacteria) that is distributed by the glassy-winged sharpshooter is highly temperature dependent and warmer winter temperatures may encourage the northern distribution of vector where in the past it was not an issue pathogen (Daugherty, Bosco, & Almeida, 2009; Hoddle, 2004; Martensson, 2007).

The same concern was predicted for several other pathogens introduced in Europe responsible for the fungal disease black rot, *Guignardia bidwellii*, or *Metcalfa pruinosa* (Maixner & Holz, 2003). However, some vectors are not favourited by climate change, such as *Scaphoideus titanus*, i.e., the responder of flavescence dorée suggests that global warming may modify the interrelationships between the vine and pest development (Stock et al., 2005). This disadvantage to them is probably since diapause termination and egg hatching require cold winter temperatures (Chuche & Thiery, 2009).

An important organism from a viticultural and oenological point of view is *Botrytis cinerea*, which is basically responsible for noble rot at best, but often for bunch rot at worst. The first one is a prerequisite to producing very expensive sweet wines in specific regions, such as Sauternes, Monbazillac, and Tokaji and a high temperature seem to contribute to its formation, but there are still several studies to be carried out before a clear conclusion can be reached on this trend (Makra et al., 2009).

2.2 Winemaking consequences

Certainly, the main microbiological and technological challenges are the higher temperatures of the harvested grapes delivered to the winery, the higher ambient temperatures during fermentation, the higher sugar in the grapes, and second but not least, the higher potassium concentrations, lower acidity levels, and higher pH values.

2.2.1 Harvest condition and fruit quality

Higher temperature and pH values during or after the harvest and initial grape processing will be observed much more frequently with the average temperature increase and advanced harvest dates, thus leading to grape damage favoured by indigenous microorganisms residing on the grapes and grape handling equipment, as well as chemical oxidation.

The uncontrolled spread of organisms can lead to several undesired consequences, such as a rapid onset of fermentation. This can reduce the effectiveness or even make cold settling impossible, i.e., pre-fermentation sedimentation to remove coarse lees, through CO_2 formation induced mixing od musts. Furthermore, the competition between indigenous organisms and yeast for nutrients may lead to sluggish or stuck alcoholic fermentation, but this can be addressed with nutrient additions or additives to counteract unwanted microorganisms, (Bayrock & Ingledew, 2004).

While the latter problem can be defeated, the production of secondary metabolites, such as acetic acid can be problematic from an organoleptic point of view and can also interfere with yeast viability and fermentation efficiency by increasing volatile acidity (Edwards, Haag, & Collins, 1998; Edwards, Reynolds, Rodriguez, Semon, & Mills, 1999; Huang, Edwards, Peterson, & Haag, 1996; Rasmussen, Schultz, Snyder, Jones, & Smith, 1995).

Further secondary metabolites, like acetaldehyde and pyruvate which combine with preservative SO_2, can pose a health problem because mycotoxins produced by this interaction and by certain fungi are particularly carcinogens (Leong et al., 2006). A study by Soleas and colleagues of 942 commercial wines (363 white wines and 580 red wines) explains the production of ochratoxin A (OTA) by Aspergillus and Penicillium. Its production is influenced by temperature and humidity and Table 2 shows that its concentration is higher in southern countries such as Spain and Portugal than in northern countries such as France (Soleas et al., 2001).

In 3.9% of the white and 16.6 % of the red wines, OTA was detectable in a concentration > 0.05 µg/L showing that the incidence is still low in white wines, while in red wines it could increase more and more as temperatures rise.

	white wines			red wines		
country or region	total	no. >0.05	%	total	no. >0.05	%
Argentina	14	2	14	17	5	29
Australia	11	1	9	45	9	20
Canada	42	0	0	54	3	5.5
British Columbia	10	0	0	18	1	5.5
Ontario	32	0	0	36	2	5.5
Chile	20	0	0	42	8	19
Central Europe	25	3	12	27	6	22
France	33	2	6	59	6	10
Greece[a]	16	2	12.5	23	4	17
Germany	16	1	6	5	0	0
Italy	36	3	8	101	16	16
New Zealand	29	0	0	10	1	10
Portugal	14	0	0	23	5	22
South Africa	18	0	0	13	2	15
Spain	6	0	0	36	12	33
United States	40	0	0	71	8	11
California	32	0	0	58	8	14
Oregon/Washington	8	0	0	13	0	0
total	362	14	3.9	580	96	16.6

Table 2 Description of Commercial Wines Analyzed with OTA Concentration >0.05 μg/L (Soleas et al., 2001)

2.2.2 High sugar and alcohol concentration

As we know sugars content determine the final alcohol content, so high sugar concentration due to a rise in the global average temperature, may cause growth inhibition or lysis in microorganisms that are turned in sluggish or stuck alcoholic fermentation, because high ethanol appears to impact plasma membrane fluidity in a complex fashion (Coulter et al., 2008), so the complete consumption of sugar by yeast during alcoholic fermentation is sometimes complicated due to excessive alcohol content. High but non-lethal sugar concentrations can produce osmotic stress in microorganisms and affect wine quality since this stress was found to regulate glycolytic and pentose phosphate pathway genes, and leads to increased formation of fermentation by-products, such as acetic acid and glycerol (Erasmus, van der Merwe & van Vuuren, 2003).

Data demonstrating the increase in alcohol in wine were provided by the Australian Wine Research Institute (AWRI) that reported an increase in the mean from 12.4 to 14.4% for red wines and from 12.2 to 13.2% for white wines between 1984 and 2008. As shown in Figure 2, these trends are largely attributable to winemakers' preference for riper grapes to obtain a wine with high fruit flavours, nevertheless with more sugar and thereby lead to higher alcohol wines, and of course, this increase is not only due to a winemaking choice but also to global warming (Godden and Muhlack, 2010).

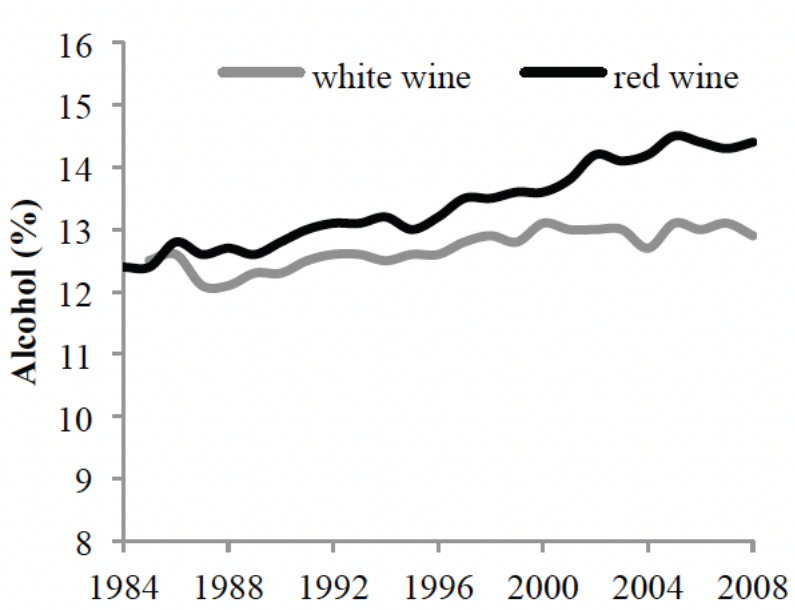

Figure 2 Mean increasing from 1984 to 2008 in Australian wines (Godden and Muhlack, 2010).

One of the reasons why high ethanol content is of concern to wine producers, in addition to health aspects and other implications for quality-related to it, is the sensory balance of the wine, because:

- Increase in the perception of astringency due to tannin, bitterness, and sourness due to acid (Martin and Pangborn 1970; Fischer and Noble 2004)
- Influencing viscosity and body
- Increase in the perception of warmth or hotness (Wilkinson and Jiranek 2013)
- Some wine aroma and flavour attributes are masked by the excess of alcohol (Le Berre et al., 2007; Robinson et al. 2009)
- In general, unbalanced wines, especially when the service temperature is high (Guth and Sies 2002; Gil et al. 2013)
- Further many high alcohol wines also express unpleasant hotness masking positive notes of fruits and freshness.

So not surprisingly, winemakers want to reduce the concentration of ethanol.

Moreover, malolactic fermentation (MLF), which is a secondary fermentation carried out by wine lactic acid bacteria mostly in red wines and some white wines, may be affected by increasing alcohol levels, and it is also difficult because of excessive volatile acidity due to acetic acid production. A slowdown in malolactic fermentation or blockage of malolactic fermentation threatens the efficiency and quality of the wine, delaying the ageing and stabilization of the wine and increasing the risk of

sensory deviations (Lonvaud-Funel, 1999). Normally, if desired, MLF is initiated in wines after the end of AF, where it can occur spontaneously by naturally occurring organisms or with an inoculum of these bacteria. Among the factors that negatively influence the start of malolactic fermentation are nutrient deficiencies, high concentrations of SO_2 or other inhibitors, and factors resulting from climate change, such as high ethanol levels which have been shown to affect the integrity of the membrane and certainly low pH values.

With the decarboxylation of malic to lactic acid and the potential production of ammonia from amino acid metabolism successful malolactic fermentation leads to an increase in pH, which can exacerbate already high pH values in wines from warm climates that have not been acid adjusted (Liu, Pritchard, Hardman, & Pilone, 1996).

Due to various inhibiting factors, difficult MLF could affect not only warm climates in the future but also cold climates, where alcohol levels would increase moderately, which in combination with high acidity would impair this type of fermentation.

2.2.3 Effects of lower acidities and increase pH levels

A tendency towards higher pH values entails the risk of increased microbial contamination, since bacterial proliferation is favoured by a pH of 4-5, and the higher the pH the lower the effectiveness of the mole fraction of SO2 as an antiseptic effect, so a low pH is a key parameter in controlling microbiological stability. In the early stages of fermentation, a high pH can lead to uncontrolled growth of lactic acid bacteria or spoilage yeasts, such as Dekkera/Brettanomyces, which can lead directly to a few organoleptic deviations during alcoholic fermentation.

Kudo, Vagnoli, and Bisson (1998) showed that a high level of potassium could be a direct factor in causing fermentation blockages in the must, especially if pH levels were low and therefore the potassium to hydrogen ion ratio was high.

Increased pH values also favour oxidative reactions and may affect wine colour, taste, and aroma. For instance, high pH promotes the development of colourless hemiketal anthocyanins form that reduces colour in young red wines (Ribéreau-Gayon et al, 1998b). Also, under a condition of high pH values, the reaction rate of acid-catalyzed hydrolysis decreases, leading to higher stability fermentation stability in high pH wines but a slower release of aromatic compounds from glycosidically bound precursors (Baumes, 2009; Williams, Strauss, & Wilson, 1980)

3. Alcohol level reduction

3.1 Viticultural Strategies

All those strategies are adopted in the vineyard to reduce the concentration of sugar in the grapes, therefore working on the initial product, without resorting to interventions on the must/wine. These include the use of unripe grapes, different viticultural techniques, the use of new varieties, new clones, and rootstocks, or simply moving the growing area to more suitable areas.

3.1.1 Use of unripe grapes

It means harvesting grapes at an early stage of ripening, during cluster thinning for example, since harvest at a very phenolic and aromatic maturity leads to obtaining high sugar and low acid concentrations, as shown above. However, this is not a good choice because grapes might not have reached an adequate maturation, which would certainly produce bitter and herbaceous wines (Canals et al., 2008; Zamora, 2014), basically, because insufficiently ripened grapes have a lower extractability of anthocyanins and proanthocyanidins from skins and a higher extractability of proanthocyanidins from seeds that are more located.
If the temperature during ripening is above the optimum threshold, the pulp of the grape ripens faster than the rest of the berry, and pH and sugar concentration become too high, leading to another issue, i.e., a shorter period between veraison and industrial maturity, making it more difficult to identify the right aromatic and phenolic maturity, and leading to unbalanced wines (Zamora, 2003, 2005).

The following study focuses on the use of grape berries harvested during bunch thinning by winegrowers who often remove a few bunches of grapes at the beginning of veraison to improve the grapevine development of the remaining grapes. The thinned bunches, which are usually left on the ground in the vineyard, can in this case be harvested and used to make a highly acidic wine with low alcohol content. After alcoholic fermentation of these grapes, this wine will be treated with a high dose of carbon and bentonite to remove phenolic compounds and the herbaceous aromas that inevitably arise from unripe grapes. Then an odourless and colourless wine will be obtained which can be used later on the wine made from the grapes left on the vine, to reduce the final pH and the and ethanol content.
During the 2008 harvest, at the beginning of veraison, the clusters of Grenache were thinned out in the experimental vineyard belonging to the Faculty of Oenology of Tarragona (Rovira i Virgili University). Were taken 500 kg of thinned grapes for the experiment where the grapes were crushed

and lightly pressed in a pneumatic press to obtain 250 L of grape juice. The must be immediately sulphited and placed in a stainless-steel tank where it was allowed for 20 hours, then racked to another tank and inoculated with selected yeast to start the alcoholic fermentation at 18 ± 1 °C.

In the end, the wine was sulphited and treated with charcoal and bentonite to obtain absolute decolouration and deodorization. The obtained wine presented the following analytic parameters: 5 % v/v of ethanol, titratable acidity of 17.8 g tartaric acid/L, and low-ethanol wine showed that absorbance at 420 was 0.023, at 520 was 0.018, at 620 was 0.013 and at 280 nm 2.56.

Afterwards, grapes of Cabernet Sauvignon, Merlot, and Bobal were harvested at two different ripening stages. The first one (H1) was executed when the potential alcohol content was between 13.0 and 14.0 %, while the second one (H2) was executed when the grapes had reached optimal phenolic maturity. For each variety, 60 kg (HI) or 120 kg (H2) of grapes were collected and distributed randomly into three lots (H1) or six lots (H2) of 8 kg each, then, the grapes were crushed and placed in 10 L tanks. Of this micro vinification, three were operated for the first harvest (H1) and three for the second (CH2) both without any addition of low ethanol wine. Instead, the other three thanks from the second harvest (RAH2) were used for the alcohol-reduction experiment, removing part of the volume, and replacing it with the same amount but of wine with low alcohol content. All tanks were inoculated with selected yeasts and maintained at 25 ± 1 °C until the end of the maceration/fermentation process. After 14 days of maceration, the wines were decanted and then transferred to bottles and inoculated with lactic acid bacteria, *Oenococcus oeni.* At the end of MFL, all the wines were sulphited and left for 3 weeks at 4 °C. Finally, all samples were stored at 15 °C until analysis.

The analytical methods recommended by OIV were carried out to determine sugar content, potential alcohol content, titratable acidity, pH, and ethanol content of wines. Furthermore, were carried out colour parameters analysis (for the intensity, lightness, chroma, hue, red-greenness, and yellow-blueness) anthocyanin analysis, proanthocyanidins, other phenolic compounds, and astringency index. To consider the sensory aspect as well, the various wines were then tasted by various expert oenologists from the Rovira i Virgili University. Standard wine parameters are shown below on Table 2 (Kontoudakis et al., 2011).

Cultivar	Parameter	H1	H2	
			CH2	RAH2
Cabernet Sauvignon	Ethanol (% v/v)	14.0 ± 0.1[a]	15.4 ± 0.1[b]	14.5 ± 0.1c
	TA (g/L)	6.50 ± 0.13[a]	5.90 ± 0.04[b]	6.72 ± 0.19[a]
	pH	3.48 ± 0.02[a]	3.55 ± 0.02[b]	3.46 ± 0.03[a]
Merlot	Ethanol (% v/v)	13.4 ± 0.1[a]	15.9 ± 0.1[b]	14.2 ± 0.1[c]
	TA (g/L)	7.00 ± 0.17[a]	6.35 ± 0.24[b]	7.15 ± 0.09[c]
	pH	3.45 ± 0.01[a]	3.76 ± 0.03[b]	3.55 ± 0.07[c]
Bobal	Ethanol (% v/v)	13.2 ± 0.1[a]	16.9 ± 0.1[b]	13.9 ± 0.1[c]
	TA (g/L)	7.45 ± 0.09[a]	6.70 ± 0.05[b]	8.94 ± 0.06[c]
	pH	3.46 ± 0.01[a]	3.80 ± 0.01[b]	3.34 ± 0.08[c]

Table 2 Key parameters (Kontoudakis et al., 2011).

The wines obtained from the first vintage (H1) naturally had higher titratable acidity, lower ethanol content, and lower pH than the corresponding control wines from the second vintage (CH2), showing how the three wines had matured between the first and second harvests.

In the table, it can be observed that all the wines substituted for the low-grade wine (RAH2) had lower ethanol content and pH than their corresponding controls (CH2). All three parameters were more similar to those of the corresponding H1 wines than to those of the corresponding CH2 wines for the three cultivars. Particularly, RAH2 wines had 0.9% (Cabernet Sauvignon), 1.7% (Merlot), and 3.0% (Bobal) lower in alcohol than their corresponding CH2 wines. These results are quite conclusive and show that replacing part of the grape juice with low-alcohol wine can be useful in obtaining wines with lower alcohol content and a lower pH.

Table 3 Indicates the total anthocyanin concentration (mg/L) determined by HPLC (Kontoudakis et al., 2011).

Cultivar	Parameter	H1	H2	
			CH2	RAH2
Cabernet Sauvignon	Anthocyanidin-3-monoglucosides	131.9 ± 11.9[a]	184.7 ± 12.5[b]	191.5 ± 21.0[b]
	Acetylated anthocyanidins	46.7 ± 6.4[a]	66.5 ± 4.5[b]	69.1 ± 8.1[b]
	p-Coumaroyl anthocyanidins	6.5 ± 1.7[a]	11.0 ± 0.6[b]	11.0 ± 1.1[b]
	Total anthocyanins	185.1 ± 32.5[a]	262.2 ± 17.8[b]	271.7 ± 30.5[b]
Merlot	Anthocyanidin-3-monoglucosides	135.7 ± 13.9[a]	187.0 ± 20.2[b]	201.9 ± 4.2[b]
	Acetylated anthocyanidins	42.3 ± 4.5[a]	47.9 ± 3.2[ab]	49.2 ± 1.2[b]
	p-Coumaroyl anthocyanidins	13.4 ± 2.0[a]	17.5 ± 1.1[b]	19.3 ± 0.4[c]
	Total anthocyanins	191.4 ± 20.2[a]	252.4 ± 24.7[b]	270.5 ± 4.8[b]
Bobal	Anthocyanidin-3-monoglucosides	241.7 ± 21.1[a]	293.1 ± 15.2[b]	320.6 ± 45.6[b]
	Acetylated anthocyanidins	18.5 ± 1.0[a]	18.9 ± 0.7[a]	19.7 ± 2.1[a]
	p-Coumaroyl anthocyanidins	18.4 ± 2.2[a]	22.5 ± 1.5[b]	24.1 ± 3.0[b]
	Total anthocyanins	278.6 ± 24.4[a]	334.5 ± 17.5[b]	364.4 ± 51.0[b]

Table 3 Anthocyanins results (Kontoudakis et al., 2011).

This table indicates that the wines from the second harvest (H2) presented higher total anthocyanin concentrations than their corresponding wines (H1), but contrary to what was expected, the anthocyanin concentration of RAH2 wines of the three varieties was founded like those of their controls (CH2). This is since a portion of extracted juice and subsequent replacement with low alcohol wine removes some anthocyanins. In addition, it has been found that a high ethanol content favours the extraction of anthocyanins during skin contact (Canals et al. 2005). A plausible explanation for these results could be the low pH of the RAH2 wines, as it seems to favour the extraction of anthocyanins during vinification, compensating for the positive effect of ethanol.

However, what producers are most interested in is whether the human eye can distinguish colour variations, rather than whether there are statistical differences.

Therefore, the total colour differences were grouped in Table 4 to determine whether the eye could distinguish between different wines (Kontoudakis et al., 2011)

Cultivar	H1 vs CH2	H1 vs RAH2	H2 vs RAH2
Cabernet Sauvignon	22.6 ± 0.8	24.7 ± 1.9	3.9 ± 1.4
Merlot	11.8 ± 3.0	25.3 ± 2.1	16.3 ± 1.4
Bobal	14.9 ± 1.9	25.6 ± 3.8	13.6 ± 3.9

Table 4 Total colour differences (ΔEab, the distance metric) (Kontoudakis et al., 2011).

The human eye can distinguish the colour of two wines only if the ΔEab* ≥ five units (Pérez-Magariño and González-Sanjose 2003). In our case, the differences in ΔEab* between H1 and the corresponding second harvest H2 (CH2 and RAH2) were greater than five units, Therefore, it can be stated that all H2 wines are distinguishable from the corresponding H1 wines. In addition, the differences between RAH2 and CH2 Merlot and Bobal were also greater than five, noting that the colour differences were distinguishable, while in the case of Cabernet Sauvignon the difference between RAH2 and CH2 was only 3.9, probably for the lower pH concerning the other cultivars.

Table 5 instead show the total phenolic compounds, proanthocyanidin concentration and the astringency index (Kontoudakis et al., 2011)

Cultivar	Parameter	H1	H2	
			CH2	RAH2
Cabernet Sauvignon	TPI	40.3 ± 1.5[a]	44.6 ± 1.8[b]	45.3 ± 5.2[ab]
	Proanthocyanidins	740 ± 100[a]	1010 ± 310[a]	1140 ± 250[a]
	Astringency index	146 ± 21[a]	171 ± 10[b]	183 ± 13[b]
Merlot	TPI	24.2 ± 2.6[a]	37.8 ± 5.2[b]	49.3 ± 1.3[c]
	Proanthocyanidins	330 ± 60[a]	1010 ± 120[b]	1140 ± 60[b]
	Astringency index	56 ± 6[a]	118 ± 42[b]	145 ± 39[b]
Bobal	TPI	35.4 ± 3.6[a]	68.1 ± 2.6[b]	63.0 ± 3.0[b]
	Proanthocyanidins	740 ± 200[a]	1650 ± 220[b]	1950 ± 400[b]
	Astringency index	96 ± 27[a]	342 ± 19[b]	204 ± 1[c]

Table 5 Total phenolic compounds, proanthocyanidins, and astringency index (Kontoudakis et al., 2011).

The more mature grapes produced more tannic wines, which is demonstrated by the data in the table stating that H2 values were higher than H1 wines. Moreover, the proanthocyanidins concentrations of the RAH2 wines of the three cultivars were quite similar to those of their corresponding CH2 wines, suggesting that replacing a fraction of the must with low-alcohol wine did not affect tannicity. However, small differences were found in the TPI and astringency index, particularly, the TPI of Merlot CH2 was lower and the astringency index of Bobal CH2 significantly higher than the respective low-alcohol wines.

Proanthocyanidins were also measured by acid catalysis as shown in Table 6 and the results were relatively comparable to those obtained in Table 5 (Kontoudakis et al., 2011)

Cultivar	Parameter	H1	H2	
			CH2	RAH2
Cabernet Sauvignon	Proanthocyanidins (mg/L)	703.6 ± 31.3[a]	1104 ± 183.4[b]	940.1 ± 197.8[b]
	mDP	4.35 ± 0.09[a]	6.54 ± 0.20[b]	6.34 ± 0.14[b]
	(+)-Catechin (%)	18.6 ± 0.4[a]	12.4 ± 0.5[b]	13.1 ± 0.4[b]
	(−)-Epicatechin (%)	56.2 ± 1.5[a]	54.6 ± 1.2[a]	55.0 ± 0.7[a]
	(−)-Epicatechin-3-O-gallate (%)	4.5 ± 0.1[a]	4.8 ± 0.4[a]	4.3 ± 0.2[a]
	(−)-Epigallocatechin (%)	20.5 ± 0.9[a]	28.2 ± 0.1[b]	28.4 ± 0.7[b]
Merlot	Proanthocyanidins (mg/L)	427.0 ± 115.5[a]	1070.1 ± 17.2[b]	969.9 ± 41.4[c]
	mDP	2.72 ± 0.13[a]	4.80 ± 1.84[b]	4.43 ± 0.36[b]
	(+)-Catechin (%)	26.2 ± 2.2[a]	21.8 ± 1.4[b]	19.0 ± 2.1[b]
	(−)-Epicatechin (%)	57.8 ± 0.7[a]	57.6 ± 0.1[a]	57.6 ± 1.9[a]
	(−)-Epicatechin-3-O-gallate (%)	4.4 ± 0.4[a]	4.9 ± 0.2[b]	5.7 ± 0.3[c]
	(−)-Epigallocatechin (%)	11.6 ± 1.3[a]	16.5 ± 0.6[b]	17.7 ± 0.7[b]
Bobal	Proanthocyanidins (mg/L)	761.4 ± 54.0[a]	1648.3 ± 38.0[b]	1573.5 ± 78.6[b]
	mDP	6.60 ± 0.14[a]	9.54 ± 0.30[b]	8.77 ± 0.34[c]
	(+)-Catechin (%)	18.9 ± 0.3[a]	11.3 ± 0.6[b]	13.2 ± 1.1[c]
	(−)-Epicatechin (%)	54.5 ± 0.8[a]	60.4 ± 0.3[b]	57.5 ± 1.0[c]
	(−)-Epicatechin-3-O-gallate (%)	3.2 ± 0.1[a]	3.6 ± 0.3[a]	3.6 ± 0.3[a]
	(−)-Epigallocatechin (%)	23.3 ± 1.0[a]	24.7 ± 0.5[b]	25.6 ± 0.2[b]

Table 6 HPLC analysis (Kontoudakis et al., 2011).

This method also had the advantage of allowing the determination of mDP and the percentage of the different monomers of proanthocyanidins. These data indicate that mDP was significantly higher in

all H2 wines than in H1 wines, which is probably because polymerization of proanthocyanidins increased with ripeness, or that the higher ethanol content favoured the extraction of larger molecules. The ripening of the grapes also influenced the production of the various proanthocyanidin monomers, e.g., the proportion of (+)-catechin was seen to decrease significantly while (-)-epigallocatechin proportion increased significantly in the three cultivars during ripening, whereas, of (-)-epicatechin and (-)-epicatechin-3-O-gallate remained nearly unchanged.

Regarding the sensory analysis of the wines, a triangular test was carried out, comparing the three wines of each variety, all represented in the following table (Kontoudakis et al., 2011).

Cultivar	Triangular test	Positive identifications	*P*	Preferences	
				Flavour	Taste
Cabernet Sauvignon	H1 vs CH2	7/10	0.05	2/5	1/6
	H1 vs RAH2	5/10	ns	1/4	2/3
	CH2 vs RAH2	4/10	ns	2/2	3/1
Merlot	H1 vs CH2	7/10	0.05	1/6	2/5
	H1 vs RAH2	7/10	0.05	3/4	3/4
	CH2 vs RAH2	3/10	ns	2/1	1/2
Bobal	H1 vs CH2	8/10	0.005	2/6	7/1
	H1 vs RAH2	9/10	0.001	4/5	8/1
	CH2 vs RAH2	9/10	0.001	4/5	9/0

Table 7 Sensory analysis (Kontoudakis et al., 2011).

These tests were effectuated by comparing the three wines of each cultivar, and as mentioned before, in some cases there were quite significant differences in colour between the wines, so to avoid influences, the analyses were carried out blind with dark glasses.

The tasters were able to distinguish the wines from the first vintage, as the H1 grapes were less ripe, from the second vintage controls. As a result, differences in ethanol, acidity, and polyphenolic composition between them were evident. In the case of Cabernet Sauvignon and Merlot, tasters showed a preference for CH2 wines over H1, both on the nose and in the mouth.

In contrast, the results for the Bobal variety were less clear. Indeed, tasters preferred the CH2 wines on account of their bitterness, while they liked H1 better overall, probably because the excessive alcohol content of the former made them too aggressive.

The experts also distinguished H1 and RAH2 in the case of Merlot and Bobal but were unable to distinguish between Cabernet Sauvignon H1 and RAH2, which could be due to differences in the maturity of the Cabernet Sauvignon grapes between the two vintages, which was lower than for the other two cultivars. Unexpected preferences were demonstrated in the case of Cabernet Sauvignon as the wines were not differentiated. Furthermore, there was a taste preference in the case of CH2 Merlot

wines. In apparent contradiction, the tasters' preference for H1 rather than RAH2 Bobal wine could be because of the excessive acidity provided by the addition of the low-grade wine
in the RAH2 wine. Finally, the tasters were only able to distinguish between CH2 and RAH2 for the Bobal cultivar, clearly because this wine had a more predominant addition of low-alcohol wine.
The conclusion can be drawn that the proposed procedure can be useful for a partial dealcoholisation and the simultaneous decrease reduction of the pH of wines, also because the colour of the wines with reduced alcohol was even better than their controls despite having similar phenolic composition, and this methodology does not require any additional equipment for the cellars as it is simply an application in the vineyard.

3.1.2 Viticultural techniques

There are many techniques to adopt in the vine, and the most obvious technique is increasing yield by enhancing the bud load, lowering cluster thinning, and choosing a vigorous rootstock.

Proper irrigation management

Increased water supply causes dilution of sugars, but on the other hand also negatively influences phenols, however, it seems that water supply from fruit set to veraison does not influence the vine to stress by limiting sugar production. (Cooley et al., 2005). In fact, among the different moments of irrigation experienced in a dry climate, the application of water only from veraison to harvest proved to reduce sugar accumulation without modifying the phenolic composition and wine quality in the Cabernet Sauvignon (Fernandez et al., 2013).
In general, the late supply of water seems to be a useful strategy to resume the shoot growth in the middle phase of sugar accumulation in the berries, making the plant concentrate on vegetative growth rather than on the accumulation of photosynthates available for the bunches.

Pruning systems

Minimal winter pruning, to significantly increase bud load and number of shoots per vine, is known to stimulate certain compensatory behaviours leading to numerous small and scattered bunches on a large canopy and well lit, with small berries richer in skin phenols than usual and therefore harvested at a lower sugar concentration (Clingeleffer, 2007)

Modulating source-sink relationship and reducing photosynthetic activity

Source-sink relationships, based on the relationship between photosynthesizing leaf area and fruit mass that attracts a large proportion of photo-assimilates, are considered of fundamental importance in modulating grape ripening and quality. For example, it is known that limiting leaf area at fruit set can reduce final berry size and improve berry composition (Ollat and Gaudillère, 1998).

However, it should be considered that either thinning of the shoots or bunches can reduce grape yield and, therefore, may increase the sugar accumulation in the ripening bunches. It should also be considered that thinning of bunches and shoots can lead to more "fruity" wines, but on the other hand can also penalise tannin extractability in some varieties (Sun et al., 2012). Changes in the leaf/fruit ratio, meanwhile, can reduce the speed of berry ripening and the final sugar content. Stoll and co-workers (2010) were able to show that topping by leaving 6 leaves per shoot could slow down the ripening of Riesling grapes by 20 days and thus reduce the final sugar accumulation by about 4 °Brix. Regarding the shoot topping technique, the practice of it carried out after fruit set induced a significant reduction in the leaf area/grape weight ratio, thus slowing down the ripening process resulting in a reduction in sugar content, bunch weight and berry weight in Grenache and Tempranillo cv. however, it also penalised the concentration of total polyphenols and anthocyanins in the must (Balda and Martinez de Toda, 2011).

Leaf removal is also effective in modulating the source-sink ratio. Indeed, leaf removal above the cluster area one month after veraison reduced the leaf-to-fruit ratio by 41 % and was shown to slow down the ripening of Sangiovese grapes and lower the sugar concentration and alcohol level of the wine (-0.6 %) (Palliotti et al., 2013). The removal of leaves after veraison in the most distal portion in the Sangiovese (removing 60 %) and Montepulciano (29 %) varieties reduced the leaf/fruit ratio at harvest by 38 % for the former variety and 16 % in the latter without affecting the weight, the total must acidity and alcohol level, but lowering the sugar concentration -0.7 °Brix in both varieties and compromising anthocyanin and polyphenol concentration in the case of Montepulciano (Lanari et al., 2013). In a study carried out in north-western Italy, in the Nebbiolo production zone, leaf removal and thus shade management positively influenced phenolic composition, but negatively influenced sugar accumulation in the berries compared to the control. (Guidoni et al, 2008; Chorti et al., 2010). Regarding the influence of the training system, Sylvoz and Lyre for increasing vigour, productivity and shading of the bunches were found to help reduce potential alcohol in Sousón variety.

The reduction of photosynthetic activity per unit leaf area can be managed by applying shade nets over the canopy, thus making the flow of photosynthetic photons on the leaf surface available. Depending on the colour and density of the net different levels of shading can be achieved.
Thus, by measuring the diurnal rates of the net CO_2 absorption by the leaves per unit leaf area in Sangiovese vines exposed to 100 %, 60 % and 30 % full sunlight, depending on colour and density, a significant difference was observed between the two extreme treatments at flowering. At harvest, vine productivity decreased by only 14%, but sugar content decreased by 23% (from 21.9 °Brix to 16.8 °Brix) (Palliotti et al, 2012).

Another method of reducing leaf CO_2 influx is a canopy spray made by distillation of conifer resins. Once the product is applied, it evaporates in a few hours leaving the leaves covered with a thin transparent layer which limits the rate of leaf gas exchange. Investigations carried out since 2008 have shown that post-veraison antiperspirant treatments can induce a significant reduction in sugar concentration and, consequently, in the alcohol level of wine, regardless of the cultivar and the productivity of the vine. However, antitranspirant treatments may induce some detrimental effects on phenolic content, especially for anthocyanins in blackberry grapes, while the total polyphenol content seems less affected, which might not be desirable for aged red wines, but might be acceptable for rosé and Beaujolais or for base wines to be blended with others richer in colour and phenolic compounds (Palliotti et al., 2008; 2010; 2013).

Plant growth regulator treatments

As we know, hormones such as abscisic acid and ethylene are known to have a direct influence on ripening processes, including colour development, while auxin levels are normally reduced when fruit ripening starts. Thus, among the techniques that aim to slow down grape ripening of grapes, the use of plant growth regulators may be proposed. A demonstration was provided by the research of Davies et al. collaborators (1997), by soaking bunches of Shiraz for 30 seconds in benzotriazole-2-oxaloacetic acid. grapes for 30 seconds in benzotriazole-2-oxaloacetic acid (BTOA) 6 and 8 weeks after flowering, and it was seen that this treatment was able to delay the evolution of physicochemical changes linked to ripening. Changes such as increased berry weight, anthocyanin, and hexose accumulation, increased abscisic acid and degradation of chlorophyll and organic acids. This is since the expression of genes typical of the pre-veraison phase continued for a long period, while the expression of genes typical of the ripening period was delayed.

Some cytokinins, e.g., CPPU (forchlorfenuron), applied in the pre-ripening phase, can reduce the total soluble solids concentration and berry skin colour; while berry weight and total juice acidity are increased (Han and Lee, 2004).
On the other hand, the application of auxin in pre-veraison (1-naphthalenacetic acid) has been shown to delay the ripening of Shiraz berries in terms of sugar accumulation in the juice and anthocyanin content in the skin, while the sensory characteristics of the wine did not change compared to the control, so applications of this hormone may be a useful technique to control the composition of the fruit (Böttcher et al., 2011).

3.1.3 New varieties, clones, and rootstocks

For centuries, grape cultivars were selected by the growers to adapt them to their wants. Native vine cultivars represent a historical and national heritage and their genetic diversity ensuring good adaptation to local environmental conditions could contribute to an adaptive response and unique must composition. Unfortunately, during the last fifty years the number of varieties in the world wine production was significantly reduced preferring the so-called noble cultivars, e.g., Cabernet Sauvignon, Merlot, Pinot Noir, Chardonnay, Sauvignon Blanc.
The reappraisal of local varieties or the selection of new clones as well as the combination of new rootstocks can help determine the genetic basis for low sugar content but the high quality of must flavour.

In the following investigation, the effect of temperature and water stress (a consequence of higher average temperatures) on different cultivars in the same research field in three consecutive years (2010, 2011, 2012) by the Research Institute of Viticulture and Oenology (RIVO) of the University of Pecs (Hungary)
All the data were obtained for each year, then the results of must and wine analysis were collected according to varieties, clones, and rootstocks, as well as field conditions.

- 2010 was an extremely wet year with 50 % more precipitation. The average temperature during the growing season was 17.4 °C, so we can state that was a cool wet year.
- 2011 the precipitation was 30 % less. The average temperature during the season was 19.1 °C, then was a good average year.
- 2012 was 40 % less than the average of the last 50 years. The temperatures were much respect last two years, at 20.3 °C. So was a warm and dry year.

As consequence, the harvest in 2010 was delayed and the must-have lower sugar content, the vintage of 2011 gave a high quality must while 2012 resulted from increased sugar content and reduced quantity of the must. Regarding the choice of the variety of vine is influenced by the site since the variety needs to be adapted to the climate of the region. The institute has got more than 1500 items of clones and rootstock, as an example here it presents the case of a clonal selection of Kardarka, a popular red grape variety in Hungary that originated from the Balkans.

This variety is a grapevine resistant to drought, having frost sensitivity and susceptibility to rot and shrivelling. It is very exploitable as a variety since it is possible to be produced white, rosé, siller (dark rosé, typical from Hungary), red and aszu (a Tokaji wine) wine from it, and these wines show up characteristic, mildly aromatic, elegant with a fresh acid content (Kozma, 1963; Németh, 1967).

From an old Kadarka (planted in 1898) that was suitable for selection, clones of high biological value were selected, a total of 56 elite strains. Based on the statistical analysis, a significant difference in morphological characteristics, as well as in vine yield and vine strain performance could be observed. In this statistical analysis, including the comparison of each clone and vintage with the other, it was shown that the values of the measured parameters were significantly influenced by the variables, justifying the diversity of the clones and vintages. Among these 56, the analysis of 16 elite strains of the highest value was continued in the second stage of selection. The sugar content of the must of the selected clone of 2010 is compared to the P. 9 control clone (ninth parcel of 16) and it is given in percentile divergence, as shown in figure 3 which illustrates the divergences in sugar content.

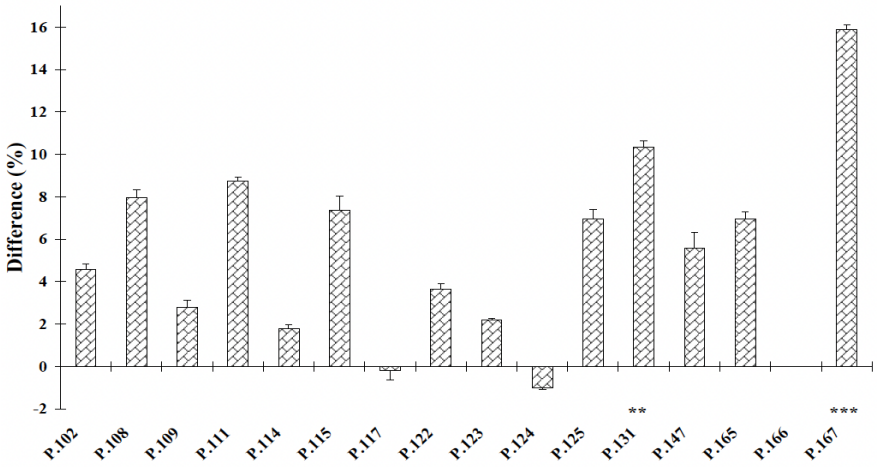

Figure 3 The divergences in sugar content pf the must of selected Kadarka clones from P- 9 clone in vintage 2010 (Jakab et al., 2013).

Concerning the harvest data of 2011 compared to the P. 9 showed significant divergence in the quantity of yield, in the average bunch weight, and the sugar content of the must. The P. 114 clone had a lower value and P. 108, P. 117, P. 125, and P. 166 clones had a higher value in the average bunch weight. The divergences in the sugar content of the musts of the selected clones were

statistically lower in the case of three selected clones (P. 109, P. 166, P. 167), the remaining clones were similar to the P. 9 clone, showed a higher value (figure 4).

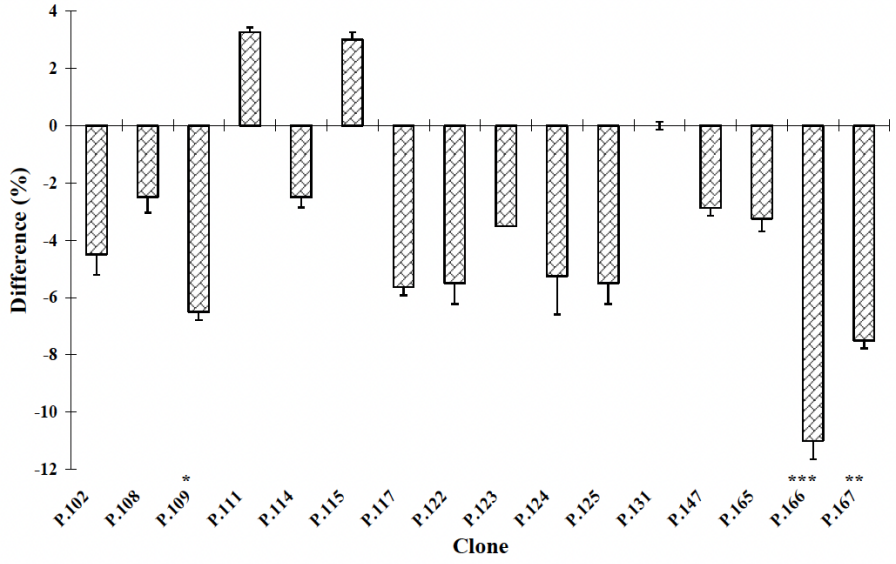

Figure 4 The divergences in sugar content of the must of selected Kadarka clones from P. 9 clone in vintage 2011 (Jakab et al., 2013).

According to the presented examples exploration of the genetic capacity of different autochthonous cultivars, clonal selection, as well as the exploitation of rootstock-scion interactions may help to answer to the new challenges.

Further research on sugar content is indicated by the results obtained with the varieties Chardonnay and Sauvignon blanc and their six different rootstocks (125AA, Fercal, Richter 110, Ruggeri 140, Teleki 5BB and 5C). Figure 5 summarises all the data obtained and it can be seen that depending on the vintage there was a strong effect on the sugar content of the musts, especially with a higher level in both varieties in the 2012 vintage

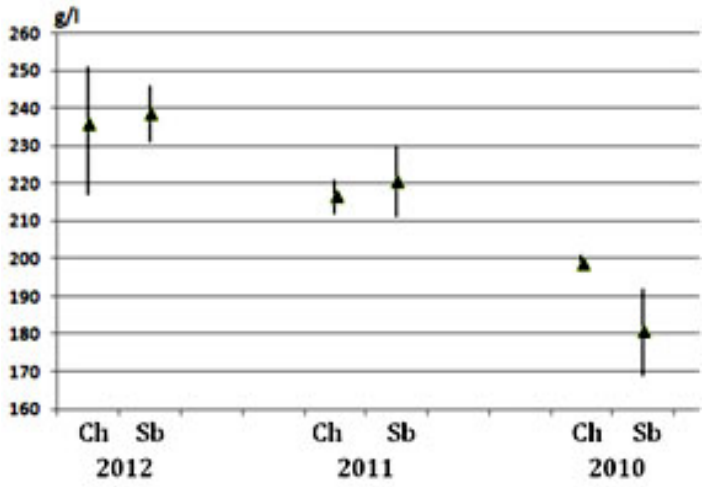

Figure 5 Average sugar content (g/l) of the musts of Chardonnay (Ch) and Sauvignon blanc (Sb) on different rootstocks at harvest in vintage 2010, 2011, 2012 (Jakab et al., 2013).

In the case of Chardonnay, the effect of the rootstocks on must sugar content was clean-cut in the year 2012, while in the case of Sauvignon Blanc there was less variation, as we can see in the columns of Table 6 (Jakab et al., 2013).

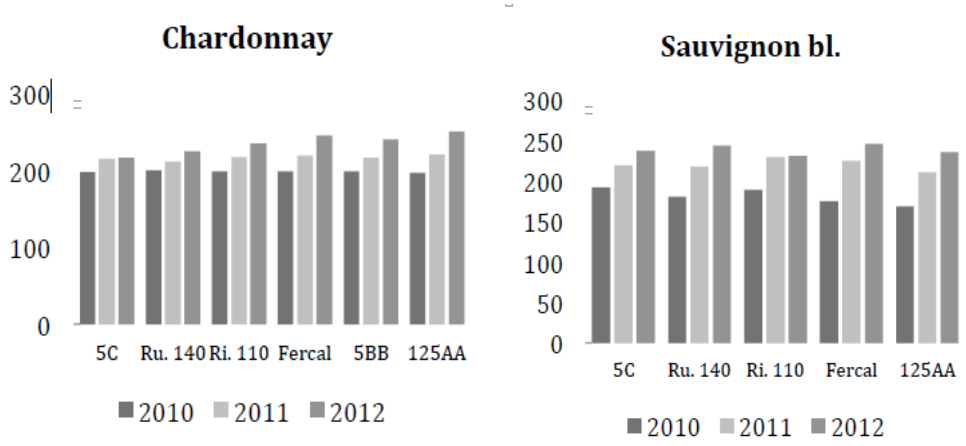

Figure 6 Sugar content (g/l of the musts of Chardonnay and Sauvignon blanc on different rootstock at harvest in vintage 2010, 2011 and 2012 (Jakab et al., 2013).

A contribution to research was also made in Australia by Clingeleffer in 2007, where low to medium vigour genotypes were proposed to produce grapes for low alcohol wines. One example is the Merbein series, which, compared to traditional rootstocks, is able to stimulate colour and phenolics improvement of about 20% in Shiraz grapes harvested at 1.5 °Brix lower than usual maturity.

This research has led to the breeding of new varieties with resistance to hot weather, which have achieved higher yields, better chemical composition and improved sensory characteristics; this is the case of Cienna a low alcohol wine released in 2000 (Clingeleffer, 2007).

3.1.4 New production areas

The main areas of grape cultivation and wine production have traditionally been located between the 30th and 50th parallels in the north and between the 30th and 40th parallels in the south, both having annual isotherms of 10°C and 20°C (Amerine et al., 1980).

An analysis of 27 wine-growing regions worldwide showed that average winter and summer temperatures increased by 1.3 and 1.48°C respectively (Jones et al., 2005a, 2005b). These data show how global warming is shifting the areas of vine cultivation. It has been estimated that the limits of cultivation in Europe are shifting northwards by 10 to 30 km per decade and that the rate is expected to double between 2020 and 2050 (Kenny and Harrison, 1992) which means that regions that are suitable for wine growing today may no longer be suitable in the future (unless appropriate measures

are taken) while regions that have so far been unsuitable for production due to their colder temperatures may become suitable in the future.

One of the earliest analyses of the impacts of climate change on viticulture was conducted by Kenny and Harrison (1992) and indicated potential shifts and/or expansions in the geography of wine regions, with parts of southern Europe expected to become too warm to produce high quality wines and northern regions becoming viable again.

Depending on the underlying scenario, climate models predict a global temperature increase of 1.5°C to > 5.0°C by the end of this century (IPCC, 2007). A temperature increase of this magnitude would substantially alter the geography of wine-growing regions with the potential for relatively large latitudinal shifts in viable wine-growing areas. In the figure below (Figure 7) we can see how favourable production areas are shifting and according to one estimate this could be the result before 2100 (Gregory V. Jones Southern Oregon University, 2007).

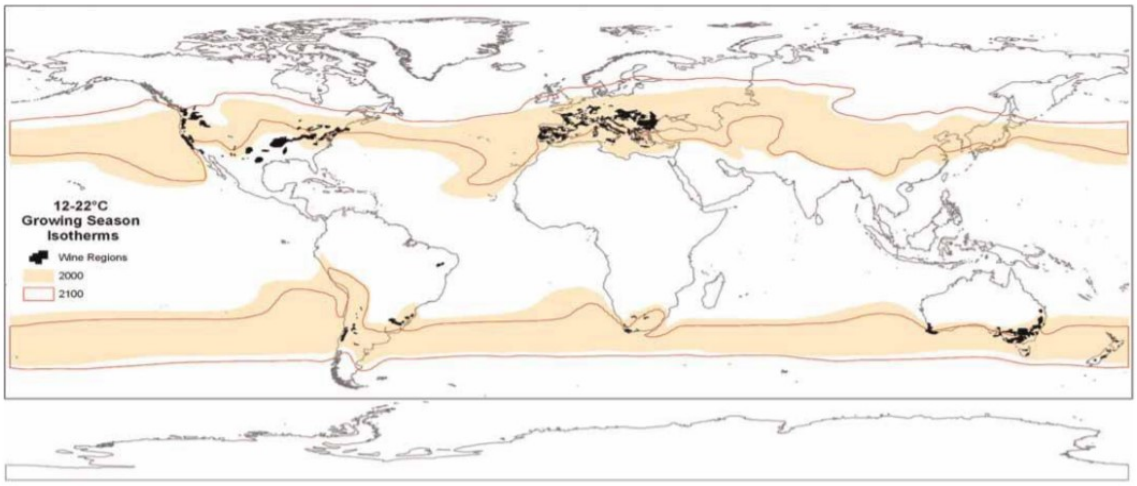

Figure 7 Map of growing season average temperatures (Northern Hemisphere April-October; Southern Hemisphere October-April) derived from observations and model runs from the Community Climate System Model (CCSM).

3.2 Membrane separation process

Previously, viticultural methods for reducing the sugar concentration in grapes have been explained, but nowadays, the most widely used methods to reduce the alcohol content in wines are physical methods (Schimdtke et al., 2012): for example, reverse osmosis or the rotating cone column, whose main objective is to obtain alcohol-free wines or wines with very low alcohol content. However, most wineries only focus on reducing the alcohol content by one or two degrees in order to obtain more balanced wines (Meillon et al., 2010a, 2010b, Gambutti et al., 2011).

3.2.1 Reverse Osmosis

It is probably the most widely used procedure for reducing the alcohol content of wine. The basic application of RO is the application of high pressure to the wine (60 to 80 bar) so that specific substances migrate through a semi-permeable membrane with a pore size of around 0.1-1nm, which retains molecules larger than a certain size, from high to low concentration. The application of such high pressures inevitably leads to temperature increases at the membrane surface, so the prevention of over-temperature during operations requires the use of auxiliary heat exchangers.

The primary fluid undergoing this treatment produces a permeate stream (water and ethanol), and a retentate stream (the remaining compounds, macromolecules, salts, etc...). There are two main RO modes applicable:

- **Dead-end**, where the feed material flows directly to the filter, the permeate passes through the RO membrane, and the retentate remains on the feed side of the membrane throughout the dealcoholisation process.
- **Cross-flow**, the feed material flows tangentially to the membrane surface, and a portion of the feed material selectively passes through the membrane (permeate). The rest of the feed material remains on the feed side of the membrane (retentate). The retentate is washed away by the influx of fresh feed material and is continuously collected on the downstream side of the reverse osmosis unit.

Special membranes are used for reverse osmosis, i.e., they are asymmetric flat-sheet membranes made of cellulose acetate or cellulose triacetate. The composite membrane with a high-strength polymer support layer is the most common, as it provides the necessary permeate rates, good selectivity and can be cleaned and rinsed to remove any contaminating material. In contrast, cellulose acetate or cellulose triacetate thin film membranes are commonly used for ethanol removal.

Operating parameters such as feed pressure, temperature and flow rates affect the effectiveness of ethanol removal and it is, therefore, necessary to determine the optimal conditions for ethanol permeation and retention of other wine components (Catarino et al., 2007).

Various membrane configurations have been developed for this purpose, including flat sheet, tubular, hollow fibre and last but not least because it is the best in terms of space, spiral wound membranes.

In these membranes, concentration polarisation is an inevitable consequence of the separation processes. This phenomenon takes the form of an increase in the concentration of molecules at the membrane level which form a polarisation layer that opposes the passage of solvent molecules (Cuperus & Nijhuis, 1993). This problem is higher in Dead-end filtration than in Cross-flow, since the fluid arrives directly at the membrane, but the problem can be solved by regular backflushing

during operations, which can alleviate the effects of membrane fouling to some extent and restore membrane performance.

The Table 8 below shows an experimental partial dealcoholisation test by reverse osmosis with two red wines from AOC Priorat and Penedès (Gil et al., 2013). The resulting test showed that only significant differences were found in alcohol content, while the other laboratory parameters remained almost unchanged.

Parameter	AOC Penedès			AOC Priorat		
	Control	-1%	-2%	Control	-1%	-2%
Ethanol content (%)	14.8 ± 0.2 A	13.8 ± 0.2 B	12.8 ± 0.2 C	16.2 ± 0.2 A	15.1 ± 0.2 B	14.1 ± 0.1 C
Titratable acidity (g/l)	4.8 ± 0.1 A	4.8 ± 0.1 A	4.9 ± 0.1 A	5.2 ± 0.1 A	5.2 ± 0.1 A	5.6 ± 0.1 B
Color intensity	15.3 ± 1.5 A	15.6 ± 0.9 A	15.4 ± 0.7 A	15.4 ± 0.2 A	15.2 ± 0.4 A	14.5 ± 0.5 A
Hue	67.7 ± 1.1 A	67.9 ± 0.4 A	68.3 ± 1.5 A	59.3 ± 1.2 A	60.0 ± 0.4 A	59.2 ± 0.5 A
Anthocyanins (mg/l)	567 ± 41 A	546 ± 19 A	574 ± 14 A	200 ± 13 A	206 ± 23 A	226 ± 11 A
IPT	72.9 ± 2.5 A	73.9 ± 2.3 A	75.8 ± 20.6 A	62.4 ± 0.5 A	62.2 ± 0.2 A	62.1 ± 0.8 A
Proanthocyanidins (g/l)	1.8 ± 0.3 A	1.6 ± 0.2 A	1.7 ± 0.2 A	1.6 ± 0.2 A	1.7 ± 0.3 A	1.5 ± 0.2 A
mDP	6.8 ± 1.2 A	7.5 ± 1.8 A	7.2 ± 0.6 A	6.8 ± 1.8 A	5.8 ± 0.3 A	6.5 ± 0.7 A

Table 8. Partial dealcoholisation by reverse osmosis (Gil et al., 2013)

The aim of this work was to study the impact of an experimental partial dealcoholisation by reverse osmosis in red wine composition and sensory characteristics (Gil et al., 2013). Specifically, two different wines from the 2009 vintage were analysed. The first wine was made from Cabernet Sauvignon grapes (AOC Penedès) and the second from Grenache and Carignan cultivars (AOC Priorat). Alcoholic and malolactic fermentation took place in stainless steel tanks and the final ethanol content of the wine was 14.8% for the first and 16.2% for the blend respectively. Subsequently, both wines were divided into three aliquots of 550 L, and stored the first without modification (control), the second was partially dealcoholised by approximately -1 by reverse osmosis and finally the third was partially dealcoholised using the same process to reduce the alcohol content by approximately -2 %. The Cabernet Sauvignon wines were aged in French oak barrels for two years, while the Grenache-Carignan wines were aged in new American oak barrels for 9 months. Finally, one sample each was taken after ageing for laboratory analysis.

The analyses consisted firstly of measuring the titratable acidity, which was measured by titrimetry using 0.1 N NaOH and bromine blue as an indicator. The pH values were determined using a pH meter and the ethanol content by ebullometry. The index of total polyphenols was analysed using a spectrophotometer by measuring the absorbance at 280 nm of a 1:100 dilution of the wine.

Wine	Parameter	Control wine	Dealcoholized wines	
			−1 %	−2 %
AOC Penedès	Ethanol[a]	14.8 ± 0.2 c	13.8 ± 0.2 b	12.8 ± 0.2 a
	pH	3.61 ± 0.01 a	3.60 ± 0.01 a	3.61 ± 0.01 a
	TA[b]	3.2 ± 0.1 a	3.2 ± 0.1 a	3.2 ± 0.1 a
AOC Priorat	Ethanol[a]	16.2 ± 0.2 c	15.1 ± 0.2 b	14.1 ± 0.1 a
	pH	3.55 ± 0.01 a	3.55 ± 0.01 a	3.55 ± 0.01 a
	TA[b]	3.50 ± 0.01 a	3.50 ± 0.01 a	3.70 ± 0.03 b

Table 8. General parameters for wine (Gil et al., 2013).

[a] *Alcohol degree (%vol.)*

[b] *Titratable acidity (expressed as g/L of sulfuric acid)*

Naturally, all the wines obtained by partial dealcoholisation contained less ethanol than their controls. Furthermore, these results were useful in understanding how reverse osmosis is suitable for the proposed objectives since the ethanol content was very close to the expected value.

Table 8 that except for AOC Priorat dealcoholised by -2 % vol, which had a slightly higher but statistically significant titratable acidity, no significant differences in pH and titratable acidity were found in the case of AOC Penedès. This is because the wine is an oversaturated solution of potassium tartrate, which immediately after alcoholic fermentation tends to precipitate over time. Consequently, the higher ethanol content in the control wines and the -1 % dealcoholised wine resulted in greater precipitation during oak ageing than in the -2 % wine. The fact that there was no such difference in the AOC Penedès wines can probably be attributed to the fact that they were partially dealcoholised when almost all the excess potassium tartrate had already precipitated.

Table 9 below refers to the total phenolic index (TPI), the total proanthocyanidin concentration and related parameters, such as the average degree of polymerisation (mDP), the percentage of procyanidins (i.e. polymeric tannins composed of gallocatechin) and the percentage of galloylation (percentage of procyanidins partially bound to gallic acid).

Wine	Parameter	Control wine	Dealcoholized wines	
			−1 %	−2 %
AOC Penedès	TPI[a]	73.9 ± 2.3 a	72.9 ± 2.5 a	75.8 ± 0.6 a
	TP[b]	1.8 ± 0.3 a	1.6 ± 0.2 a	1.7 ± 0.3 a
	mDP	5.96 ± 0.02 a	6.11 ± 0.09 a	5.87 ± 0.17 a
	%PC[d]	70.06 ± 0.39 a	69.33 ± 0.43 a	69.42 ± 0.73 a
	%PD[e]	29.94 ± 0.39 a	30.67 ± 0.43 a	30.58 ± 0.73 a
	%Gal[f]	3.20 ± 0.16 a	3.24 ± 0.20 a	3.48 ± 0.19 a
AOC Priorat	TPI[a]	62.4 ± 0.5 a	62.2 ± 0.1 a	62.1 ± 0.8 a
	TP[b]	1.6 ± 0.2 a	1.7 ± 0.2 a	1.2 ± 0.3 a
	mDP[c]	4.60 ± 0.24 a	4.73 ± 0.04 a	4.69 ± 0.07 a
	%PC[d]	77.00 ± 0.06 b	76.92 ± 0.32 b	75.51 ± 0.42 a
	%PD[e]	23.00 ± 0.06 a	23.08 ± 0.32 a	24.49 ± 0.42 b
	%Gal[f]	3.98 ± 0.02 a	4.21 ± 0.38 ab	4.63 ± 0.02 b

Table 9. Total phenolic content by spectrophotometry and proanthocyanidin analysis using HPLC–DAD (Gil et al., 2013)

[a] *Total polyphenolic index*

[b] *Total proanthocyanidins expressed as g/L*

[c] *Mean degree of polymerization*

[d] *Percentage of procyanidins*

[e] *Percentage of prodelphinidins*

[f] *Percentage of galloylation*

No statistically significant difference was found in TPI, TP (total concentration of proanthocyanidins) or mDP (their main degree of polymerisation) between the control and partially dealcoholised wines in AOC Penedès. Furthermore, no differences were found in the percentages of procyanidins, prodelphinidins and galloylation. Results with little variation were also found in AOC Priorat wines, although some slight but statistically significant differences were observed in the last three parameters.

The colour parameters were obtained by spectrophotometric measurements by adding twenty microliters of a 10% acetaldehyde solution to 2 mL of wine sample to avoid sulfite interference, then measurements were taken after a 20-minute incubation period. Colour intensity was estimated using the method described by Glories in the 1984. The other parameters (CIELAB* coordinates) represented in the following table (Table 10) such as brightness, chroma, hue, red-greenness and yellow-blueness were determined according to Ayala et al. (1997).

Wine	Parameter	Control wine	Dealcoholized wines	
			−1 %	−2 %
AOC Penedès	CI[a]	15.3 ± 1.5 a	15.6 ± 0.9 a	15.4 ± 0.7 a
	C*[b]	55.8 ± 1.9 a	56.3 ± 0.7 a	55.9 ± 0.2 a
	L*[c]	40.6 ± 3.2 a	40.4 ± 1.3 a	40.8 ± 0.5 a
	h*[d]	16.8 ± 0.9 a	18.1 ± 0.9 a	17.5 ± 1.3 a
	a*[e]	55.4 ± 1.1 a	53.5 ± 0.3 a	53.4 ± 0.1 a
	b*[f]	16.1 ± 1.2 a	17.5 ± 1.1 a	16.8 ± 1.2 a
AOC Priorat	CI[a]	15.4 ± 0.2 a	15.2 ± 0.4 a	14.5 ± 0.5 a
	C*[b]	55.3 ± 0.2 b	52.4 ± 0.5 a	52.7 ± 0.1 a
	L*[c]	37.9 ± 0.4 a	39.8 ± 1.2 ab	40.3 ± 0.1 b
	h*[d]	11.5 ± 0.1 b	11.1 ± 0.4 ab	10.5 ± 0.2 a
	a*[e]	52.3 ± 0.2 b	51.4 ± 0.3 a	51.8 ± 0.1 ab
	b*[f]	10.7 ± 0.1 b	10.1 ± 0.5 ab	9.6 ± 0.2 a

Table 10. Colour parameters (Gil et al., 2013).

[a] Color intensity

[b] Chroma

[c] Lightness

[d] Hue

[e] Green/red color component

[f] Blue/yellow color component

*CIELAB (CIE is the International Commission on Illumination)

For AOC Penedès wines, no statistically significant differences were found in any of the colour-related parameters. On the other hand, some slight but statistically significant differences were found in the AOC Priorat wines compared to their control, such as the data referring to chroma and brightness, which indicate that the control wine has a slightly more intense colour than the partially dealcoholised wines.

Table 11 represents a comparison between the wines to see if these small differences can be seen by the human eye. To do this, the total colour difference (ΔEab*) was calculated, which is nothing other than the difference between the various CIELAB coordinates, finding an average ΔEab*≥1. However, tasters can only distinguish the colour of two red wines through the glass when ΔEab*≥5 and since our parameter was not greater than five units, the effect of reverse osmosis on the colour of the wine was not sufficient to be distinguished by the human eye.

	AOC Penedès	AOC Priorat
Control vs. −1 %	2.37	2.19
Control vs. −2 %	2.13	2.69
−1 vs. −2 %	0.81	0.81

Table 11. Mean values of the total colour differences (DEab) between the samples (Gil et al., 2013).*

Regarding phenolic compounds, anthocyanins was analysed by HPLC-DAD (Gil et al., 2013)., which consists of directly injecting the wine into a chromatographic system consisting of a column crossed by a controlled flow of liquid eluent, after which the various chemical species making up the wine are separated as they travel through the column and detected by the spectrophotometer (DAD). In the Table 12 below are shown HPL-DAD data.

Wine	Parameter	Control wine	Dealcoholized wines	
			−1 %	−2 %
AOC Penedès	Non-acylated anthocyanins	153.3 ± 15.5 a	151.2 ± 1.3 a	158.6 ± 1.1 a
	Acetylated anthocyanins	68.7 ± 5.7 a	65.5 ± 3.5 a	67.9 ± 3.3 a
	p-Coumarylated anthocyanins	8.4 ± 1.3 a	8.2 ± 0.2 a	8.6 ± 0.1 a
	Total anthocyanins	230.3 ± 22.5 a	224.9 ± 5.1 a	235.1 ± 4.5 a
AOC Priorat	Non-acylated anthocyanins	21.2 ± 2.6 a	20.1 ± 1.7 a	31.5 ± 4.6 b
	Acetylated anthocyanins	12.6 ± 1.8 a	14.4 ± 4.8 ab	19.3 ± 0.4 b
	p-Coumarylated anthocyanins	0.6 ± 0.1 a	0.7 ± 0.1 a	1.1 ± 0.1 b
	Total anthocyanins	34.5 ± 4.5 a	31.5 ± 6.7 a	51.9 ± 5.1 b

Table 12. Anthocyanins quantification determined by HPLC–DAD, expressed as milligrams of malvidin-O-3- glucoside per liter (Gil et al., 2013).

Firstly, the concentration of total anthocyanins in AOC Priorat was very low, but this is reasonable considering that these wines were aged for 9 months in oak barrels, where the micro-oxygenation that occurs in these barrels can oxidise the anthocyanins and can influence the formation of more complex components, some of which may subsequently precipitate. The anthocyanin composition of the -1% AOC Priorat wine was statistically almost similar to that of the control, while the concentration of the -2% wine was statistically much higher than that of the control and the -1% dealcoholised wine, possibly since the first two cases had greater precipitation of tartrate salts than the -2%, which has a higher titratable acidity. The AOC Penedès control wines and the corresponding partially dealcoholised wines had statistically similar concentrations of total anthocyanins, as well as the concentrations of both acylated (acetylated and *p*-coumarylated) anthocyanins were similar.

The results of the polysaccharide analysis are shown in Table 13, which were extracted by precipitation with acidified absolute ethanol and analysed by high-resolution size exclusion chromatography (HRSEC) using a refractive index detector (RID) (Gil et al., 2013)

Wine	Parameter	Control wine	Dealcoholized wines	
			−1 %	−2 %
AOC Penedès	LMWf[a]	85.2 ± 10.1 a	84.1 ± 8.6 a	86.1 ± 4.7 a
	MMWf[b]	187.2 ± 5.7 a	222.7 ± 18.9 ab	237.8 ± 7.8 b
	SMWf[c]	233.8 ± 78.5 a	211.8 ± 19.6 a	221.8 ± 28.5 a
	Total	506.2 ± 82.8 a	518.6 ± 47.1 a	545.7 ± 41.0 a
AOC Priorat	LMWf[a]	74.6 ± 20.2 a	65.9 ± 0.7 a	73.3 ± 4.5 a
	MMWf[b]	202.9 ± 14.8 a	170.2 ± 10.5 a	218.0 ± 19.7 a
	SMWf[c]	200.1 ± 7.4 a	167.1 ± 27.5 a	225.7 ± 14.6 a
	Total	477.5 ± 27.7 ab	430.2 ± 37.4 a	516.9 ± 38.8 b

Table 13. Polysaccharide analysis by HRSEC-RID, expressed as milligrams of polysaccharide per liter (Gil et al., 2013).

[a] Large molecular weight fraction, including polysaccharides greater than 50 KDa

[b] Medium molecular weight fraction, including polysaccharides between 10 and 50 KDa

[c] Small molecular weight fraction, including polysaccharides up to 10 KDa

It was found that the total polysaccharide concentration and molecular weight fractions of the -1 % wines were like those of the control wines in both designations. While the -1 % test showed this, some slight differences appeared in the -2 % wines which generally had higher values in all fractions. This tendency may be due to the fact that the solubility of polysaccharides is lower when the ethanol concentration is higher. The table also describes that some polysaccharides in wine can inhibit the growth of potassium hydrogen tartrate crystals and therefore it is logical that wines with a higher concentration of polysaccharides have a higher titratable acidity. This demonstration was found in the case of AOC Priorat wine at 2% ethanol but not in AOC Penedès wines at 2% ethanol, probably because the latter was partially dealcoholised when almost all the excess potassium tartrate had

already precipitated. These data demonstrate the fact that dealcoholisation by RO should be applied as soon as possible to avoid the usual losses of titratable acidity that occur during cold stabilisation.

Finally, the sensory analyses of the wines were carried out in the tasting room of the Faculty of Enology of Tarragona (Rovira i Virgili University) by a group of eleven experts.

For each wine, six triangular sensory tests were carried out to compare the three wines, each using official ISO tasting glasses. The triangular tests were carried out at two different serving temperatures: 16-18 and 24-26 °C. The low temperature was considered to be the optimal serving temperature at which the characteristics and aromas of the red wines were revealed. Conversely, the higher temperature favoured the evaporation of ethanol and was chosen to reproduce the incorrect serving conditions that sometimes occur. In all cases, the main objective was to determine whether the tasters were able to recognise whether the wine was different. The second objective was to determine which of the tasters' preferred wines was the one that correctly identified different wines. However, this latter test was only carried out at the optimum serving temperature (16-18 °C). No preference test was carried out at 24-26 °C because there was such a presence of ethanol that it was difficult to appreciate the other attributes.

Table 14 shows the result of sensory analysis.

Wine	Triangular test	T (°C)	Positive identifications	*P*	Preferences		
					Control	−1 %	−2 %
AOC Penedès	Control vs. −1 %	16–18	9/11	0.005	7	2	–
		24–26	8/11	0.03			
	Control vs. −2 %	16–18	7/11	0.05	5	–	2
		24–26	4/11	ns			
	−1 vs. −2 %	16–18	1/11	ns	–	0	1
		24–26	4/11	ns			
AOC Priorat	Control vs. −1 %	16–18	4/11	ns	3	1	–
		24–26	3/11	ns			
	Control vs. −2 %	16–18	5/11	ns	3	–	2
		24–26	7/11	0.05			
	−1 vs. −2 %	16–18	5/11	ns	–	3	2
		24–26	7/11	0.05			

Table 14. Sensorial analysis results (Gil et al., 2013).

At a serving temperature of 16-18°C, nine out of eleven tasters were able to distinguish the control wines from the -1 % AOC Penedès wines. From their tastings, seven of the tasters preferred the control wine, while the other two preferred the -1 % dealcoholised wine. The results were similar when the control was compared with the wine at -2 % at 16-18 °C, seven out of eleven were able to distinguish between them, of these, five preferred the control while the other two preferred the dealcoholised. The results were more surprising when this triangular test was performed at 24-26 °C, as only four tasters were able to distinguish the control from the -2 % dealcoholised, while between the control and the -1 % dealcoholised eight distinguished them, probably this because the excessive ethanol content in both wines at high temperatures can cause tasters to become tired and saturated.

The tasters were then able to distinguish between the two dealcoholised wines but had difficulty distinguishing them at both serving temperatures, as can be seen in Table 7. It, therefore, appears that the tasters can distinguish the control wine AOC Penedès from its corresponding partially dealcoholised wines and that they prefer it, but they stated at the end of the analysis that it was not easy to distinguish between the different wines and that they had doubts about choosing their preferred wine. In the case of AOC Priorat wine, the results were less clear than in the case of AOC Penedès wine. In the comparative analysis between the control and the -1% dealcoholised, only four tasters out of eleven distinguished between the two wines at 16-18°C, while only three out of eleven did so at 24-26°C. The panel between the control and the -2% at 16-18°C also failed to provide a satisfactory result, as only five out of eleven tasters distinguished between the two wines. However, in the tasting at 24-26°C, seven out of eleven tasters were able to distinguish the two wines. The comparison between the two dealcoholised wines was identical to the previous comparison.

The results indicate that the application of reverse osmosis to the partial dealcoholisation of wine does not appreciably alter the colour or chemical composition, with the sole and intended decrease in ethanol content. Furthermore, a panel of trained tasters as seen had serious difficulty distinguishing between control and partially dealcoholised wines in triangular trials. This technique, therefore, can be very useful for the partial dealcoholisation of red wine, also because the cost of the process can be considered affordable as the equipment manufacturer provides the service at 0.15 €/L for the removal of 1% ethanol. Reverse osmosis is, therefore, an interesting tool especially nowadays as climate change is increasingly causing a mismatch between pulp maturity and phenolic maturity of the grapes (Gil et al., 2013).

3.2.2 Nanofiltration

This technique differs mainly from RO in that the pore size of the membrane is larger (approximately 0.5-5 nm).

While reverse osmosis can remove the smallest solute molecules, in the range of ≤0.0001 μm in diameter, nanofiltration removes molecules in the range of 0.001μm (Ferrarini et al. 2001).

RO has severe membrane fouling and high energy consumption; these limitations could be solved by using NF membranes.

Nanofiltration can be used to remove alcohol from wine as an alternative to reverse osmosis, but it can also be used to enrich musts derived from inadequately matured grapes with sugars (Pati et al. 2014) or to remove excess sugars when the opposite occurs (Bes et al. 2010).

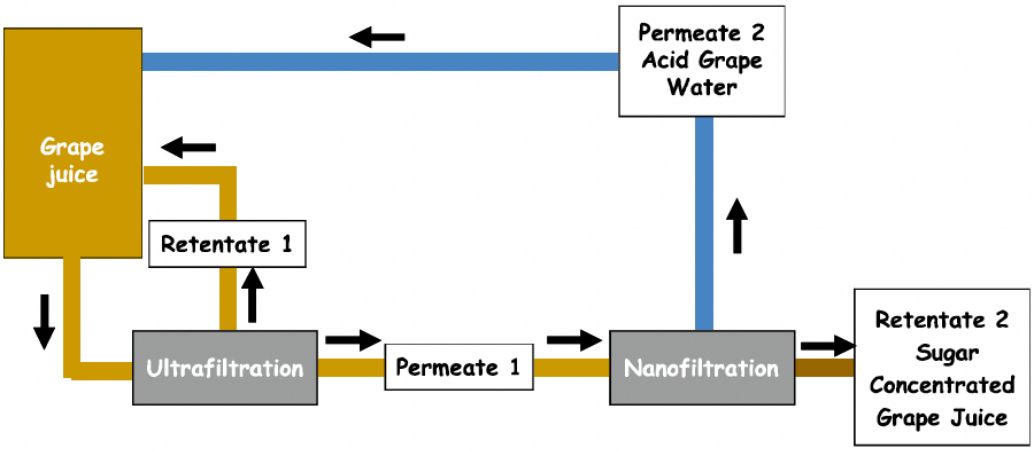

Figure 8. Illustration of sugar removal by nanofiltration (Zamora, 2016).

To filter by nanofiltration, a first ultrafiltration step is necessary to obtain the clarity required for the subsequent steps. The first retentate obtained by crossflow ultrafiltration is redirected with the original must, whereas the permeate is directed to the nanofiltration equipment. Afterwards, nanofiltration provides a new retentate that contains nearly all the sugars, and a permeate containing the grape juice water with some of their acids. This technique, therefore, allows the sugar content of the must (and the alcohol in the wine) to be increased or reduced as required: by adding water if necessary to reduce the sugar content in the must, or by increasing the sugar concentration otherwise by adding must or sugar where possible. However, the use of nanofiltration or reverse osmosis to reduce sugars in grape juice has a major financial disadvantage.

In the research below the use of nanofiltration for the reduction of alcohol levels in wine is shown and then compared to reverse osmosis. The flow chart of the equipment used for NF and RO dealcoholisation assays is presented in Figure 9.

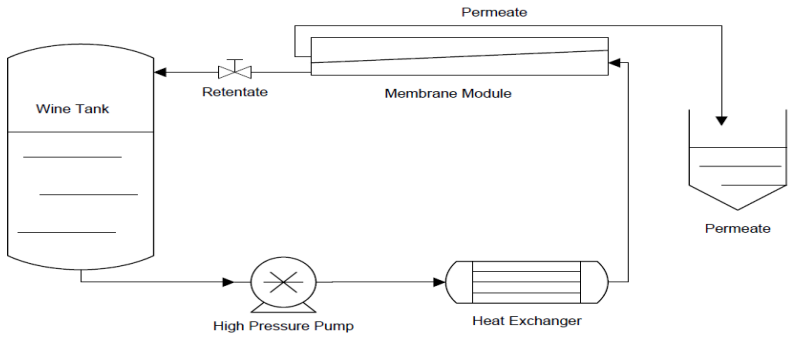

Figure 9. Scheme of the experimental set-up used for RO and NF assays (Gonçalves et al., 2013).

As we can see in the picture, the wine is first pumped from a tank employing a high-pressure pump and passes through a heat exchanger where it is cooled with cold water for temperature control. It then enters the two membrane modules wound in a spiral in series, and here we obtain two flows, one of retentate which is recirculated in the wine tank and another of permeate which is continuously removed from the complex. The difference between the two tests was determined by the fact that for reverse osmosis, a Vaslin-Bucher type X membrane at 70 bar transmembrane pressure was used, whereas for nanofiltration, the operation was carried out using Alfa-Laval ALNF97 membranes at 30 bar transmembrane pressure. The analyses carried out were first of all the calculation of the alcohol concentrations, determined by ebulliometry for levels above 4% vol and for levels lower by distillation/oxidation and titration with ferrous ammonium sulphate as described by Ribéreau-Gayon et al. (1972). According to the methods prescribed by the International Organisation of Vine and Wine (OIV) (2009), analyses were carried out on pH (OIV-MA-BS-13), total acidity (OIV-MA-AS313-01), tartaric acid (OIV-MA-AS313-05A), malic acid (OIV-MA-AS313-10) and volatile acidity (OIV-MA-AS313-02). Finally, Unicam, UV4, UV/Vis spectrophotometer was used to obtain total phenol measurements and colour characterisation as specified by Somers and Evans (1977).
A red wine with a high alcohol content of 16% was used for the tests, from which two samples were taken and dealcoholised with RO and NF, aiming for a final alcohol content of 14%. The analytical results of the original and the dealcoholised wines are presented in Table 15 (Lança, 2011).

	Original	Dealc. NF	Dealc. RO
Alcohol (%)	16.0	14.2	14.1
Total acidity (g/L)	5.87	5.55	5.81
Volat. acidity (g/L)	0.80	0.69	0.82
pH	3.39	3.38	3.33
Color intensity	10.9	11.5	12.1
Hue	0.60	0.57	0.58
Total anth. (mg/L)	404.3	366.2	298.8
Total phenols (AU)	59.8	58.0	58.0

Table 15. Comparison between the same wine dealcoholised by NF and RO (Gonçalves et al., 2013).

What we can see from the results is the fact that there is a reduction in most of the analytical parameters measured. The reduction in total acidity is more pronounced in the wine treated with NF (5%) than with RO (1%). Similarly, volatile acidity also decreased more in NF-treated wine (14%). This is related, as expected, to the fact that NF membranes allow a greater passage of ions, especially acetate ions, than RO. The total phenolic content is slightly reduced in both, but an important loss of anthocyanins is noted, especially in the RO-treated wine (26%) compared to the NF-treated wine (9%). Nevertheless, intensity of colour and tonality of the wine remain almost unchanged, On the

contrary, there is even an increase in the intensity of the colour in treated wines that is probably related to the decrease in pH. This loss is certainly not due to permeation through the membrane since its molecular mass is very high, but the more likely cause would appear to be adsorption on the membrane surface. Indeed, this insight was provided by the coloured solution obtained during membrane cleaning, which indicates the presence of phenolic material adsorbed on the membrane. Finally, sensory evaluation was carried out on wines treated by NF and RO in which no differences in colour were revealed. The results of the sensory evaluation of the wines showed a preference the NF-treated wine over the original wine and the RO-treated wine.

The use of nanofiltration was also carried out to compare two rose wines, a low-alcohol wine (7% alcohol) and a dealcoholised wine (<0.5% alcohol). In order to obtain a wine with these low alcohol values, the NF treatment was more intensive, i.e., more passes were made through the membrane and therefore more pronounced changes in the wine were expected. The analytical results of the two different rosé wines are presented in Table 16 (Ribeiro, 2007; Lemperle, 2010).

	Low-alcohol [1]		Dealcoholized [2]	
	Original	Dealc.	Original	Dealc.
Alcohol (%)	14.7	7.2	11.8	0.3
Total acidity (g/L)	4.0	3.7	4.7	3.6
Volat. acidity (g/L)	0.38	0.25	0.36	0.06
pH	3.56	3.41	3.29	2.99
Color intensity	3.36	3.33	2.47	2.39
Hue	-	-	0.69	0.64
Total anth. (mg/L)	159.9	131.9	184	149
Total phenols (AU)	16.7	13.7	15.9	12.4

Table 16. Analytical results of wo rosé wines dealcoholized by NF (Gonçalves et al., 2013).

The results in Table 2 show for both wines a reduction in total and volatile acidity, and it can be deduced that the reduction in these two parameters is more pronounced when the alcohol reduction increases, probably due to the greater number of passages and the consequent permeation of acetic acid. It can be seen that the reduction in volatile acidity is very pronounced, being 34% for the low alcohol wine test and 86% for the dealcoholised wine test.

As found in the results of Table 1, there is also a reduction in total phenols and anthocyanins in the treated wines. In contrast to the results concerning the decrease in acidity, the reduction of total phenols and anthocyanins seems to be independent of the extent of NF treatment. The observed reduction of total phenols was 18% for low alcohol wine and 22% for dealcoholised wine. For

anthocyanins, the observed reductions were 18 % for the low-degree wine and 19 % for the dealcoholised wine, which means that the values for colour intensity and hue remained almost unchanged in these trials as well. A sensory evaluation was also carried out in this case, in which the dealcoholised wines showed no significant changes in colour and aroma profile. The differences found were more related to the absence of alcohol and the wines were found to be more acidic despite the reduction in measured acidity. Overall, the dealcoholised wine was compared to commercial samples of dealcoholised wines and showed a better evaluation.

In conclusion, according to the results obtained, it can be stated that nanofiltration as a technique for the removal of alcohol from wine has proven to be effective. Compared to reverse osmosis, it has some advantages such as reduced volatile acidity and less loss of anthocyanins. In addition, nanofiltration also allows higher alcohol removal, enabling the production of low-alcohol and alcohol-free wines.

3.2.3 Pervaporation

It is a method of separating mixtures of liquids by partial evaporation through a membrane, in which the substance passing through the membrane changes its phase state, which is why it is called pervaporation. The pervaporation membrane is a non-porous membrane and can be hydrophilic or organophilic depending on its material, i.e. materials based on cellulose acetate or polyvinyl alcohol for the first variant, or composed of polyoctylmethylsiloxe, polydimethylsioxane or polytrimethylsylypropane in the case of organic substances (Catarino et al, 2009). Water molecules diffuse faster through the membrane and organic solvent particles cross the membrane more quickly, regardless of the volatility of the compounds. With this procedure, the energy requirement of the separation can be considerably reduced. In addition, it can selectively extract ethanol from wine containing thermolabile aromatic compounds. It produces no waste material, and its by-products can be used as required (wine distillate and water). Pervaporation can produce a permeate that is more concentrated in alcohol, so the wine becomes less dense than other filtrations. Conversely, aromas are also organic compounds and therefore significant aroma losses can occur when pervaporation is applied to wine. The performance of pervaporation is affected by several factors including permeate pressure, feed concentrations, interactions between feed components, mass transfer resistance and most importantly temperature (Karlsson et al., 1995). Pervaporation is a technique that can work at low or ambient temperatures, although most alcohol removal procedures have been conducted at temperatures around 30°C (Tan et al., 2005; Takács et al., 2005).

In the first phase of the article, pervaporation was carried out under laboratory conditions with the aim of producing a quality end product and providing data to model the process.
In the second phase, on the basis of the results obtained previously, the technical-economic and operational scale of the processes and the cost estimation were carried out (Takács et al., 2005).
Quality Tokaji wine of the 1997 vintage (from Tolcsva) was used, characterised by an alcohol content of 13.11% vol, the glucose concentration was 14.11 g/l; the density at 20 °C was 1004 kg/m^3, and the viscosity at 20 °C was 1.1x10^{-1} Pa. The investigations were carried out using an apparatus, shown in Figure 10 and built by the Faculty of Food Science at Corvinus University in Budapest.

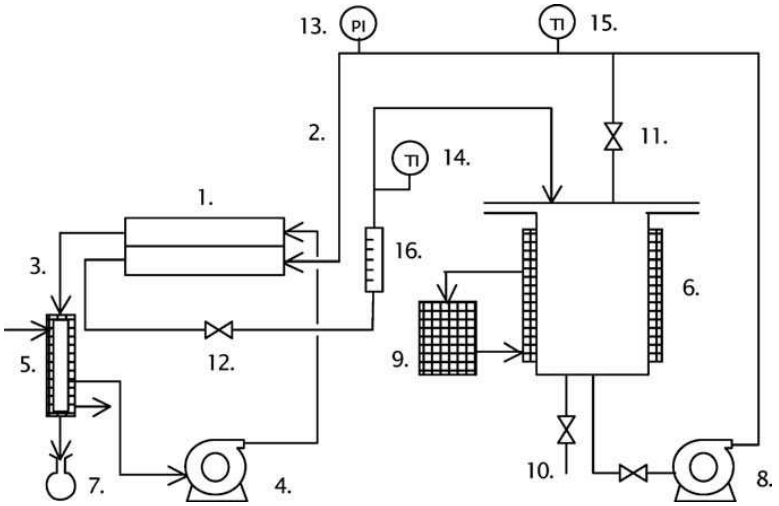

Figure 10. Laboratory scale pervaporation equipment (1) membrane, (2) liquid mixture inlet, (3) permeate vapour, (4) vacuum pump, (5) condenser, (6) liquid tank, (7) insulated permeate collector, (8) pump, (9) thermostat, (10) outlet valve, (11) flow control valve, (12) pressure control valve (13) pressure gauge, (14, 15) thermometers and (16) flowmeter (Takács et al., 2005)

The pressure applied was atmospheric as the pervaporation experiments were performed in "carrier gas mode" by recirculating air (inert gas) with a dry vacuum in a cycle where the permeated vapour was condensed. The process lasted 10 h and at the end, both the retentate volume and the total amount of permeate produced and its alcohol content were measured. The presence of wine aroma components in the pervaporation products was examined by gas chromatography and mass spectrometry. The wine aroma components and membrane separation products were extracted by distillation. After distillation of 50 cm^3 of sample alcohol, another condensed sample of 50 cm^3 was collected and the procedure was repeated twice more. The ethanol flux and the total flux of the other compounds can be derived from the permeate flux at different temperatures and the measured ethanol concentrations, as is shown in Table 17.

t (°C)	J (kg/m^2 h)	x_{PE} (v/v%)	J_E (kg/m^2 h)	J_V (kg/m^2 h)
40	0.287	38.506	0.111	0.176
50	0.548	37.640	0.206	0.342
60	0.829	36.112	0.299	0.530
70	1.200	35.125	0.421	0.779

Table 17. The average flux of permeate, alcohol content, the flux of alcohol, and other compounds determined at different temperatures (Takács et al.,2005).

The figure shows the relationship between flow and temperature, and it can be seen that the flow increases exponentially in all three cases and can be described by an Arrhenius-type equation (Rautenbach, 1997):

$$J = J_0 \cdot e^{-\frac{E_0}{R \cdot T}}$$

From these data we obtain a graph shown by Figure 11 that shows us the slopes of the straight lines that give the value of the membrane activation energies (E_0), while the intercepts determine the pre-exponential factors (J_0) that have no physical meaning.

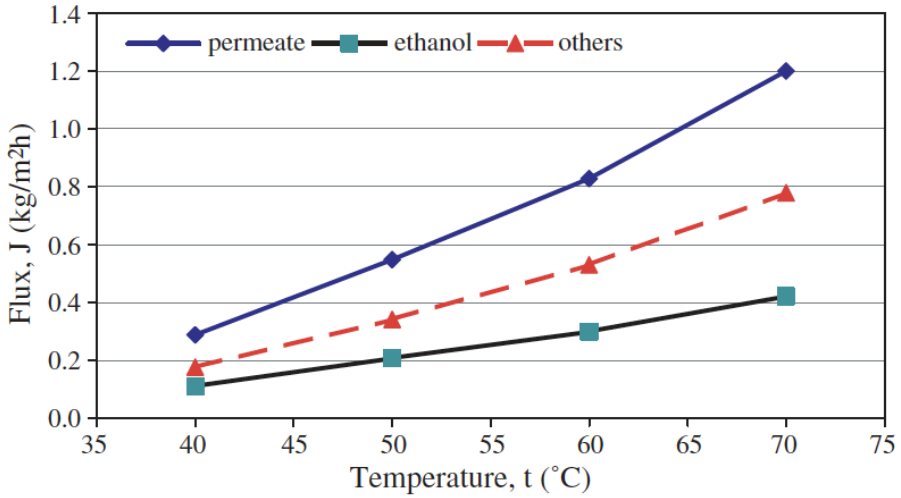

Figure 11. The exponential relationship between flux and temperature (Takács et al., 2005).

The activation energies of the membrane is the main measure of its mass transfer by evaporation accompanied by a phase change (Xianshe & Huang, 1996). The values are shown in Table 18, however, its exact determination due to the complexity of the mass transfer phenomenon is difficult.

Product	E_0 (kJ/mol)	J_0 (10^6 kg/m^2 h)
Permeate	41.36	2.38
Ethanol	39.27	0.42
Others	43.80	3.82

Table 18. The activation energies and the pre-exponential factors (Takács et al., 2005).

The table below (Table 19) sets out the second part of the test, the cost estimate.

Alcohol free wine	V_R	3	m^3
Alcohol content of alcohol free wine	x_{RV}	0.05	v/v%
Daily wine inlet	V_F	4.7	m^3
Starting concentration of ethanol	x_{FE}	13.11	v/v%
Permeate flux	J	0.292	kg/m^2 h
Volume of permeate produced	V_P	1.7	m^3
Ethanol content of permeate	x_{PE}	36.21	v/v%
Membrane surface demand	A_M	82.62	m^2
Price of complete PV-equipment	C_{PV}	315207	€
Investment costs of membrane	B_M	31521	€/year
Other costs of PV-equipment	B_E	25217	€/year
Total investment costs	$\sum B$	56738	€/year
Estimation of operational cost			
Operational costs of heating	E_F	14048	€/year
Operational costs of cooling	E_H	51840	€/year
Operational costs of pumping	E_{SZ}	25920	€/year
Total operational costs	$\sum E$	91808	€/year
Total costs	$\sum B + \sum E$	148546	€/year

Table 19. Results of Pervaporation cost estimation (Takács et al., 2005).

The results of this investigation show that temperature plays the most important role in the production of low-alcohol and alcohol-free wines by pervaporation. Therefore, the choice of the optimal temperature seems to be the main objective to optimise this process. At higher temperatures, the permeate flux is higher and the membrane surface demand is lower, thus leading to an economic advantage of the operation. However, at higher temperatures the separation efficiency of the membrane and the separation capacity decrease, so the permeate production becomes faster, but less desired product is obtained from the separation.

At high pervaporation temperatures, most of the organic compounds in the wine evaporate and end up in the condensate due to steam permeation. In order to avoid serious flavour losses due to evaporation, the choice of lower temperatures is the most advantageous. It should also be considered that the operation involves a large demand for investment costs, which can be explained by the

relatively high price of non-porous pervaporation membranes, but still the investment could be remunerated in a few years.

3.2.4 Osmotic Distillation (Perstraction)

This is a promising membrane-based technique for low to moderate rates of ethanol removal from beverages with minimal effect on the organoleptic properties of the product. The OD separation process can be treated as a liquid/liquid extraction between two liquid phases that are “virtually” immiscible with one another. Conventionally, the liquid phase from which the solute (wine or hydroalcoholic solution) is to be separated is called the "feed" and its derivatives are called "raffinates", while the liquid phase (water) that receives the volatile solute is called the "solvent" or "extractant" and its derivatives are called "extracts". In OD, the raw material is circulated through a hydrophobic hollow-fibre membrane contactor with the stripping liquid flowing on the opposite side of the membrane. In ethanol removal from wine, the stripping liquid is water degassed with hydrophobic membranes used to create the pressure differential (Varavuth et al., 2009; Diban et al., 2013). Two streams flow through a hollow-fibre hydrophobic membrane contactor, with the volatile compounds moving from the high vapour pressure liquid into the low vapour pressure liquid. The microporous hydrophobic membranes create a vapour gap between the two liquid phases. Volatile compounds from the feed solution (at high concentration) are free to migrate, by convection or diffusion, to the stripping solution, preventing the aqueous solution from penetrating the pores (Diban et al., 2008). Actually, it is not a distillation so according to I.U.P.A.C. (International Union of Pure and Applied Chemistry, 1996) it is defined as perstraction. The application of this closed-loop technique without downstream permeate treatment can clearly lead to the removal of ethanol from the wine up to the desired ethanol reduction (Hogan et al., 1998). Replacing the stripping water and pooling the DO process can lead to the complete removal of ethanol from the wine, although significant aroma losses occur when the wine is treated in this way (Liguori et al., 2013). The most common uses of this technique are the concentration of fruit juices (Varavuth et al., 2009) and the removal of ethanol from wine or beer (Hogan et al., 1998; Liguori et al., 2013). Figure 12 illustrates how Osmotic distillation can be used to reduce the potential alcohol content shows the whole process of osmotic distillation in a simple way.

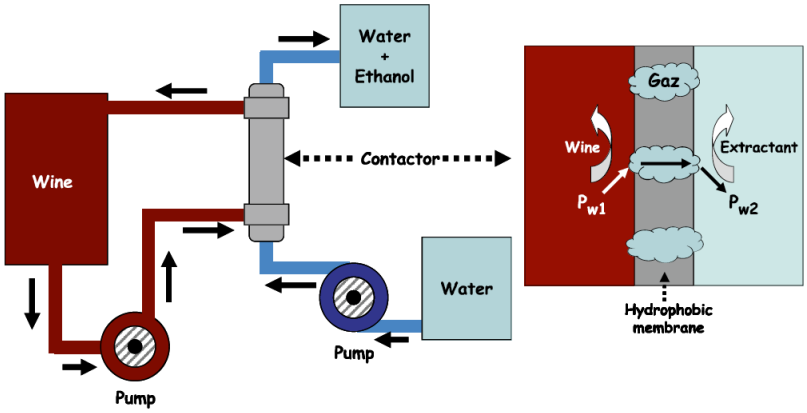

Figure 12. Osmotic distillation process (Zamora, 2016).

Single-stage batch extraction is the simplest configuration that can be achieved as it is direct osmotic distillation, which involves feeding the reactor containing the hydrophobic membrane with complete recirculation of the extracts and raffinate until the concentration of the raffinate reaches the desired value. Figure 13 shows a schematic representation of a direct one-stage OD.

The batch mode means that both the extract phase (extract) and the phase to be extracted (raffinate) are recirculated until predetermined alcohol is reached.

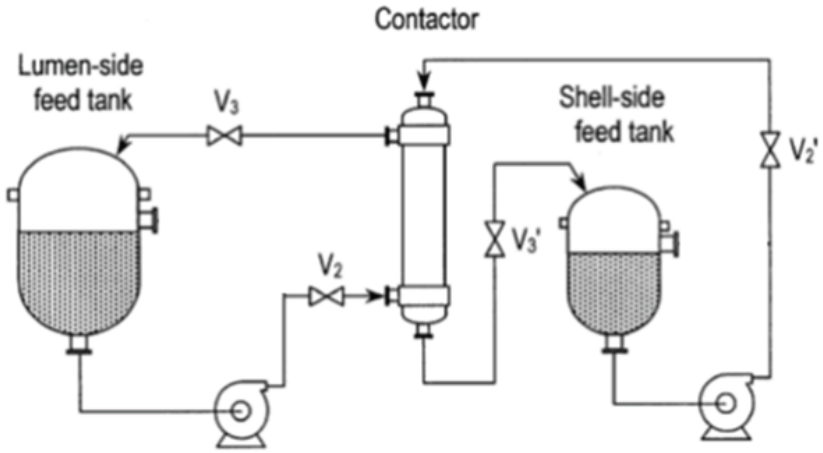

Figure 13. Single stage plant by Osmotic Distillation (Stassi et al., 2013).

Direct OD offers the following advantages: the unit is simple to construct, the process is cost-effective because no heat exchange or pressurisation is required, the product is not subjected to mechanical, thermal or chemical stress. In addition, the membrane operates under ideal conditions at low temperature and low pressure and can also operate with an alcohol content of >17%. On the other hand, the system has the following disadvantages: the efficiency of the process depends on the alcohol content of the wine and the extraction water and is medium-low, although, if correctly sized, it is

possible to achieve high extraction averages with values even higher than 10l/h of absolute alcohol and with limited water consumption. At the end of the process, the concentration of the extraction water never exceeds 6.5% v/v of alcohol, and there is also a significant risk of oxygenation of the wine (Stassi et al., 2013).

3.3 Spinning Cone Column (SCC)

The SCC is a multistage strip column initially developed in the USA in the 1930s and later modified in Australia (Gray 1993; Pickering 2000). Essentially it is a vertical column containing a rotating vertical central shaft fitted with upward-facing cones alternating with sets of fixed downward-facing conical baffles attached to the column casing. It is characterised by a counterflow system containing a succession of alternating rotating and stationary metal cones. The following picture shows the above mentioned equipment (Figure 14).

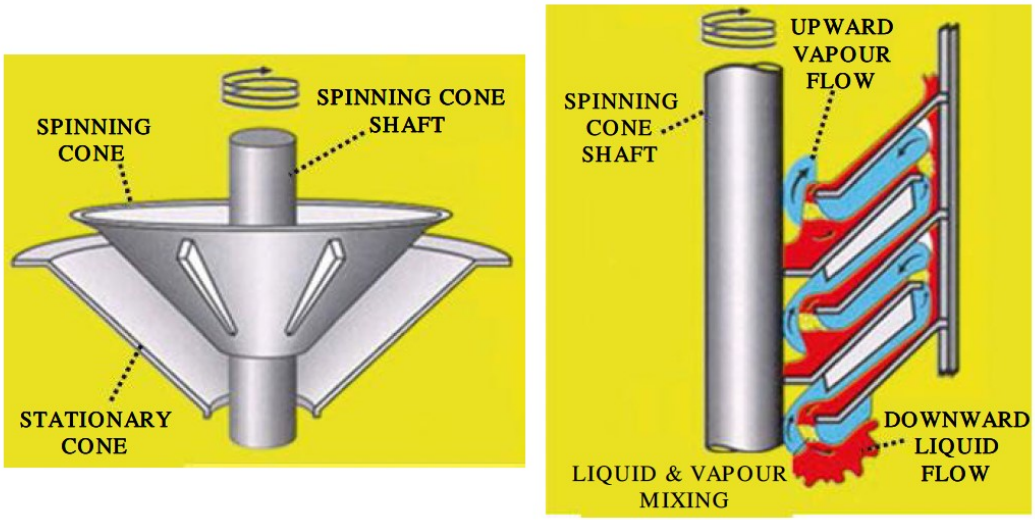

Figure 14. Spinning Cone Column (Zamora, 2016).

The liquid feed enters at the top of the column and flows down, while the stripping gas (steam or inert gas) is fed into the base of the column and flows up (Pyle, 1994). As the liquid flows downwards, a thin film is created by the centrifugal force of the rotating cone and the liquid migrates to the top of the of the rotating vane whereupon it drops onto the underneath fixed cone and migrates back towards the centre of the column. This process increases the surface of the liquid a great deal, which favours the evaporation of this volatile components. The SCC operates in vacuum, so the volatile aroma components are transferred to the gas phase in a relatively high vacuum and at low temperatures (Saha et al. 2013). Figure 15 shows the operating diagram of the Spinning Cone Column.

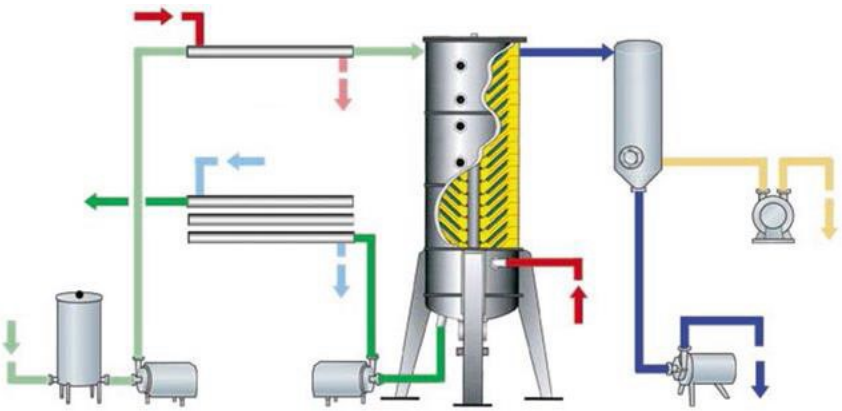

Figure 15. Operating diagram (Zamora, 2016).

The ethanol removal from wine using this technique is made in a two-stage process. In the first one, the more delicate aroma components are removed at moderate to high vacuum (0.04 atmosphere) and low temperature (26-28 °C). In the second stage, ethanol is removed from the base wine, and this stage is conducted at a higher temperature, around 38 °C, and leads to alcohol contents typically above 50% v/v (Belisario-Sánchez et al. 2009). It is possible to reduce the ethanol concentration from 15 % v/v to less than 1 % v/V using the SCC.

Finally, the final dealcoholised wine is obtained, which is produced by mixing the recovered aroma with the wine obtained from the second step of the operation (Pyle, 1994).

The spinning cone column also includes a system for recovering the volatile aroma that evaporates during the dealcoholizing process. A number of ancillary devices are required for the SCC, namely heat exchangers to warm the product feed to operating temperatures, pumps and condensers to collect the gaseous vapour and collect the removed fraction. In terms of cost, the SCC has a relatively high requirement for capital outlay and operating expense and is in fact recommended as a suitable technology for large wineries, with the added benefit of high productivity, flexible operating conditions, hygiene and application to the production of juice concentrate.

3.4 Must Replacement and Hot Pre-Fermentative Maceration

These techniques have been proposed to reduce the alcohol content without affecting the polyphenolic component of wine, i.e., by increasing it, the colour being the first sensory property to be appreciated by consumers (González-Neves, Favre, Gil; 2014). The characteristics of this parameter that dominate are clarity and intensity, which are responsible for influencing the consumer's choice, also affecting the sensory perception of aroma, taste and mouthfeel. The key players in this parameter are obviously anthocyanins, which are synthesized by the secondary metabolism of the vine and accumulate in the

grape skins during ripening. In the European vine (Vitis vinifera), i.e., the vine used to produce wine grapes, the grape anthocyanins are delphinidin, cyanidin, petunidin, peonidin and malvidin, as well as acyl derivatives with acetic, o-coumaric and caffeic acids (Alcalde-Eon, Escribano-Bailón, Santos-Buelga, Rivas-Gonzalo; 2006).

Climatic conditions are of great importance regarding the quality of the grapes and thus of the final wine. In a winemaking process, only 40% of the anthocyanins in the grapes are transferred to the wine (Boulton; 2001). This is because the lack of permeability of the cell walls and cytoplasmic membranes does not allow the release of these substances which are found in the upper layers of the skin. The extraction of these, however, can be facilitated by the simultaneous development of maceration and alcoholic fermentation, because the ethanol content leads to the disintegration of the vacuolar membranes and cell walls of the skin.

The colour of wine depends not only on the concentration of anthocyanins but also on the structural transformation they undergo from the pH of the medium. In an acid medium, they are red in colour, while the closer you get to neutral pH, they take on a purple colour and decrease in intensity as the pH increases. The closer you get to neutral pH, the more violet they turn and the more intense their colour becomes as the pH increases until they are irreversibly destroyed at excessively high pH. In addition, the colour can change during the production, storage and ageing of wine, due to the formation of new compounds and their polymerisation by modifying the colour (Fulcrand, H.; Dueñas, Salas, Cheynier; 2006) In recent decades, several alternative maceration techniques have been proposed that allow a differentiated extraction of phenolic and aromatic compounds from grapes into wine to improve quality and ageing potential.

In Uruguay, Tannat is the most important red cultivar due to its adaptation to eco-physiological conditions. This variety has grapes with a low anthocyanin extraction capacity and lower proportions of malvidin and acetylated glycosides than other red cultivars, such as Cabernet Sauvignon and Merlot. Consequently, the colour stability of Tannat wines is lower than other varieties. The high temperatures recorded in recent years and the resulting thermal stress during the ripening period cause degradation and inhibition of anthocyanin accumulation. Currently, there is a growing concern on the part of winemakers to have tools to regulate the ethanol content, pH and concentrations of phenolic compounds without causing damage to the colour of Tannat red wines, as the intensity and tone of the colour of this variety determine its target market and commercial value (González-Neves, Franco, Barreiro, Gil, Moutounet, Carbonneau; 2007).

The following research studies the impact of must replacement and pre-fermentative hot maceration on the colour of Uruguayan Tannat red wines produced in three consecutive vintages. Both techniques were used to produce red wines with lower alcohol content and pH and a higher concentration of

phenolic compounds. Pre-fermentative hot maceration involves leaving the grapes in containers in heated tanks for a variable period, promoting the degradation of the cellular structures of the grape skin. Must replacement, on the other hand, consists of replacing a percentage of very mature grape must with the juice of unripe grapes before alcoholic fermentation to reduce the alcohol content and pH of the wines (Piccardo et al., 2019).

The following research was carried out with Tannat grapes in 2016, 2017 and 2018 vintages. The grapes were harvested manually from a vineyard in the south of Uruguay. At the beginning of the veraison period, 100 kg of grapes were harvested with the aim of obtaining a must with high acidity and low sugar concentration. These grapes were crushed and lightly pressed to obtain 50 L of unripe must and then stored in a container at 4°C. The remaining grapes left on the plant were left until they reached technological maturity, and of this 120 kg of grapes were harvested and randomly distributed in 12 batches of 10 kg. The grapes were also destemmed and crushed and then distributed into 12 containers of 10 L each. Of these 12, six containers were considered as controls (original must, OM) while in the other six containers (must in the other six containers (substituted must, MR), 3 L of original grape must be replaced by 3 L of unripe must to reduce the sugar content and pH. Subsequently, three containers of OM and three of MR were macerated traditionally (TM), while the other three were subjected to hot prefermentative maceration (HM) for 1 hour at a temperature between 60 and 70 C. At the end of the treatment, the tanks were immersed in a cold-water bath to cool them down to room temperature (approximately 26 C). Once cooled, the must be transferred to the original 10-litre containers, resulting in four experimental groups as seen in Figure 16: control wine with traditional maceration (OM-TM), must replacement and traditional maceration (MR-TM), control wine with hot prefermentative maceration (OM-HM), must replacement and hot prefermentative maceration (MR-HM), control wine with hot maceration (MR-HM).

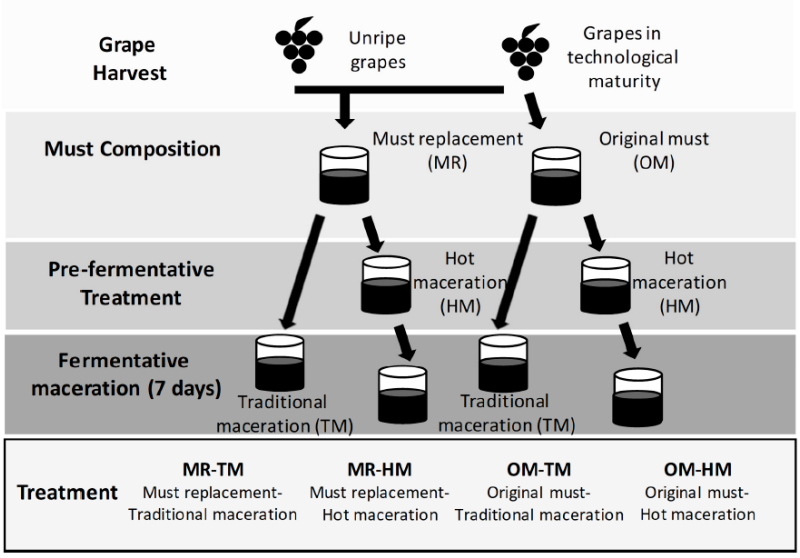

Figure 16. Process diagram (Piccardo et al., 2019).

All containers were inoculated with yeast and fermented in contact with the skins. After 7 days of maceration, the wine was separated from the marc by gravity, and the marc was lightly pressed.

The fermentation temperatures were between 26 and 29 °C in 2016, 22 and 27 °C in 2017, 25 and 29 °C in 2018. Alcoholic fermentation was completed when daily must density measurements were less than 998 g/L for three consecutive days. The wines were then stored in containers of 5 L capacity at room temperature and malolactic fermentation was carried out, which lasted approximately 35 days. Finally, the wines were bottled and stored in a dark space at room temperature. After two months, analysis was performed.

The following table (Table 20) shows the effects of different vintages, must composition and the various techniques have employed on the ethanol content, titratable acidity, pH, sugar residue and volatile acidity of the various wines.

Factor Analyzed		Ethanol (% *v/v*)	Titratable Acidity (gH_2SO_4/L)	pH	Residual Sugars (g/L)	Volatile Acidity (gH_2SO_4/L)
Year of vintage (*)	2016	14.0 ± 0.1^{b}	4.30 ± 0.27^{a}	3.92 ± 0.16^{a}	1.47 ± 0.41^{c}	0.36 ± 0.07^{b}
	2017	11.2 ± 0.2^{c}	2.93 ± 0.05^{c}	3.86 ± 0.04^{c}	1.85 ± 0.21^{b}	0.43 ± 0.09^{a}
	2018	15.4 ± 0.2^{a}	3.85 ± 0.03^{b}	3.89 ± 0.09^{b}	2.44 ± 0.44^{a}	0.44 ± 0.07^{a}
Must composition (**)	OM	14.0 ± 0.1^{a}	3.51 ± 0.17^{b}	3.95 ± 0.09^{a}	2.07 ± 0.59^{a}	0.43 ± 0.09^{a}
	MR	13.0 ± 0.1^{b}	3.88 ± 0.06^{a}	3.83 ± 0.09^{b}	1.83 ± 0.39^{a}	0.39 ± 0.08^{b}
Maceration technique (***)	TM	13.3 ± 0.2^{b}	3.74 ± 0.19^{a}	3.87 ± 0.09^{a}	2.01 ± 0.55^{a}	0.47 ± 0.06^{a}
	HM	13.7 ± 0.1^{a}	3.64 ± 0.04^{a}	3.92 ± 0.09^{a}	1.89 ± 0.46^{a}	0.35 ± 0.05^{b}
Must composition - Maceration techinque (****)	OM-TM	14.0 ± 0.2^{a}	3.61 ± 0.30^{b}	3.92 ± 0.09^{b}	2.30 ± 0.56^{a}	0.50 ± 0.06^{a}
	MR-TM	12.6 ± 0.2^{c}	3.87 ± 0.09^{a}	3.81 ± 0.12^{d}	1.72 ± 0.37^{c}	0.45 ± 0.06^{b}
	OM-HM	14.0 ± 0.1^{a}	3.40 ± 0.03^{c}	3.98 ± 0.08^{a}	1.84 ± 0.53^{bc}	0.36 ± 0.05^{c}
	MR-HM	13.4 ± 0.1^{b}	3.88 ± 0.04^{a}	3.85 ± 0.09^{c}	1.95 ± 0.39^{b}	0.33 ± 0.05^{c}

Table 20. General composition of the wines (Piccardo et al.,2019).

Wines produced from the 2018 vintage had the highest ethanol content, and those from the 2017 vintage had the lowest. Regarding titratable acidity and pH, the highest values were recorded in wines produced in 2016 and the lowest in 2017. Grapes harvested in 2016 and 2018 had better ripening conditions, with high sugar concentrations and optimal pH while, on the contrary, in 2017, grape ripening stopped, resulting in lower sugar concentrations and pH. The wines produced from 2016 and 2017 presented residual sugar concentrations below 2 g/L, while vintage 2018 showed a slightly higher value, which is probably related to a higher concentration of non-fermentable sugars.

The volatile acidity of wines produced in different vintages was predicted according to the vinification system used. The must composition factor refers to all wines produced with original must (OM) or replacement must (MR), regardless of vinification system, vintage or maceration technique. As expected, the MR wines had a lower ethanol content and pH and a higher titratable acidity than

the OM wines. Residual sugars were not much affected by the substitution of unripe must, while volatile acidity was slightly lower. With regard to ethanol content, HM wines had a higher content than TM wines, without significant differences in total acidity or pH.

The phenolic composition of the wines is shown in Table 21. It can be seen that the highest concentrations of total polyphenols, anthocyanins and proanthocyanidins were found in 2016, while the wines of the 2017 vintage had the lowest values. With regard to the concentrations of catechins, the wines produced in 2018 were undoubtedly higher than in the other vintages. The anthocyanin content in the 2018 vintage was not significantly different from that of 2016, while the concentrations of total polyphenols and proanthocyanidins were intermediate to the values of 2016 and 2017.

Factor Analyzed		Total Polyphenol (mg/L)	Anthocyanins (mg/L)	Catechins (mg/L)	Proanthocyanidins (mg/L)
Year of vintage (*)	2016	2479 ± 252 ^{a}	1052 ± 156 ^{a}	1769 ± 455 ^{b}	4172 ± 714 ^{a}
	2017	1624 ± 68 ^{c}	614 ± 68 ^{b}	1420 ± 58 ^{c}	2690 ± 60 ^{c}
	2018	2140 ± 43 ^{b}	1165 ± 43 ^{a}	1883 ± 86 ^{a}	3260 ± 80 ^{b}
Must composition (**)	OM	2045 ± 140 ^{a}	960 ± 67 ^{a}	1667 ± 239 ^{a}	3397 ± 372 ^{a}
	MR	2117 ± 102 ^{a}	994 ± 73 ^{a}	1714 ± 160 ^{a}	3352 ± 197 ^{a}
Maceration technique (***)	TM	1784 ± 112 ^{b}	838 ± 69 b	1281 ± 215 ^{b}	2764 ± 261 ^{b}
	HM	2379 ± 129 ^{a}	1117 ± 71 ^{a}	2100 ± 184 ^{a}	3985 ± 308 ^{a}
Must composition-Maceration techinque (****)	OM-TM	1821 ± 131 ^{c}	832 ± 69 ^{c}	1273 ± 268 ^{b}	2792 ± 352 ^{b}
	MR-TM	1747 ± 94 ^{d}	843 ± 69 ^{c}	1289 ± 161 ^{b}	2735 ± 170 ^{b}
	OM-HM	2345 ± 149 ^{b}	1088 ± 66 ^{b}	2061 ± 209 ^{a}	4001 ± 390 ^{a}
	MR-HM	2413 ± 109 ^{a}	1146 ± 77 ^{a}	2141 ± 159 ^{a}	3968 ± 225 ^{a}

Table 21. Phenolic composition (Piccardo et al., 2019).

These results could be explained by the fact that pre-fermentative hot maceration results in low water evaporation. Water that could have contributed to the low concentration of all compounds in the must, particularly sugars. The last factor expresses the parameters of all wines in the four experimental groups. The ethanol level of OM-TM and OM-HM wines was significantly higher than that of MR-TM and MR-HM wines, which showed differences due to different processing techniques.
In contrast, the ethanol content of MR-HM wine was significantly higher than that of MR-TM wine. In the results, we can see that MR-TM and MR-HM wines were found to have higher titratable acidity values and lower pH than OM-TM and OM-HM wines. Considering the use of the two techniques in the same wine, changes in pH were observed, associated with the initial composition of the must and the maceration technique. In this respect, it was reported that wines developed through pre-fermentative hot maceration showed higher pH values because, during pre-fermentative heating, cation extraction increases and therefore pH increases. In addition, wines produced by must replacement and/or treated by hot pre-fermentative maceration showed the lowest values in both sugar and volatile acidity. The total polyphenols, anthocyanins, catechins and proanthocyanidins of the

wines compared to the must composition parameters, there were no major differences between the MR wines and the results of the OM wines. Techniques to reduce the alcohol content of the wines reduced the concentration of highly polymerised flavonols without substantially changing the concentration of anthocyanins, partly because the substitution was done before maceration. In contrast, for the two maceration techniques, the total polyphenols, anthocyanins, catechins and proanthocyanidins of the HM wines were significantly higher than those of the TM wines, confirming that this technique can be useful for polyphenol extraction, as the pre-fermentative heating helps to degrade skin tissue. When we analysed the joint effect of grape juice composition and maceration technique, it was observed that wines produced by pre-fermentative hot maceration had the highest concentrations of the different phenolic families evaluated. In this respect, HM-OM wines were found to be lower in total polyphenols and anthocyanins than HM-MR wines, which was not the case for catechins and proanthocyanidins, where no significant differences were found.

Factor Analyzed		Color Intensity	Lightness (L*)	Chroma (C*)	Hue (h_{ab})
Year of vintage (*)	2016	32.5 ± 1.4^{a}	31.5 ± 1.2^{b}	45.0 ± 1.0^{b}	348.1 ± 1.6^{a}
	2017	16.0 ± 0.5^{c}	60.5 ± 1.5^{a}	28.1 ± 1.5^{c}	10.6 ± 1.3^{c}
	2018	24.2 ± 0.5^{b}	25.5 ± 0.9^{c}	53.1 ± 0.8^{a}	11.8 ± 0.5^{b}
Must composition (**)	OM	23.2 ± 0.9^{b}	40.2 ± 1.3^{a}	41.0 ± 1.2^{b}	3.27 ± 1.0^{a}
	MR	25.1 ± 0.8^{a}	37.9 ± 1.2^{b}	43.1 ± 1.4^{a}	3.74 ± 1.2^{a}
Maceration technique (***)	TM	20.4 ± 0.7^{b}	44.8 ± 1.2^{a}	41.4 ± 1.3^{b}	4.66 ± 0.8^{a}
	HM	27.9 ± 0.9^{a}	33.3 ± 1.3^{b}	42.7 ± 1.3^{a}	2.35 ± 1.4^{a}
Must composition - Maceration techinque (****)	OM-TM	19.6 ± 1.0^{d}	45.9 ± 1.4^{a}	40.4 ± 1.0^{c}	5.11 ± 0.6^{a}
	MR-TM	21.2 ± 0.4^{c}	43.8 ± 0.9^{b}	42.6 ± 1.7^{b}	4.21 ± 0.9^{a}
	OM-HM	26.8 ± 0.8^{b}	34.6 ± 1.2^{c}	41.6 ± 1.5^{bc}	1.43 ± 1.4^{c}
	MR-HM	29.0 ± 1.1^{a}	32.0 ± 1.4^{d}	43.7 ± 1.0^{a}	3.27 ± 1.5^{b}

Table 22. Colour parameters (Piccardo et al., 2019).

Table 22 shows the colour parameters of the wines produced. We can see that the 2016 wines had the highest intensity of colour and the greatest hue, while those produced from the 2017 vintage presented the highest brightness and the lowest intensity, chroma and hue. The 2018 vintage provided wines with the highest chroma value, but instead with intermediate values between 2016 and 2017 in colour intensity and hue. In general, the MR wines had a deeper red colour because the colour intensity, chroma and hue were significantly higher and the brightness was lower than in OM wines, while HM wines also had a deeper colour than TM wines because the colour intensity and chroma were significantly higher and the brightness was significantly lower in HM wines. When analysing the effect of the initial must composition and maceration technique, it was observed that MR-HM wines presented the highest colour and chroma intensity and the lowest brightness, while OM-TM wines

presented the lowest values. OM-HM wines presented the lowest hue value compared to the others. RM-HM and OM-TM wines considering the other parameters presented intermediate values.

The differences in the colour parameters of the wines were associated with the differences in the concentrations of phenolic compounds found, those of anthocyanins, from which extraction increased by the pre-fermentative hot maceration, thus explaining the differences in the parameters. In addition, the increased extraction of tannins allowed a greater association of anthocyanins, which was reported to be a determining factor in improving colour stabilisation (Cheynier, Dueñas-Paton, Souquet, Sarni-Manchado; 2006).

While it is true that in a sensory evaluation, the colour characteristics of these wines can be difficult to distinguish, even for a group of experts, it must be considered that the wines were evaluated two months after bottling. As the wine was stored for two months before analysis, it is well known that the colour of wine evolves during storage and the results obtained in this research suggest that wines produced using both winemaking techniques may have a more stable colour over time and, consequently, greater ageing potential. Replacing ripe grape juice must with unripe grape juice and pre-fermentative hot maceration are technological alternatives to improve the colour of Tannat red wines. As we could see, the effect of HM on the colour and the final wine depends very much on the composition of the grapes. In contrast, HM improved the intensity and quality of wine by increasing the extraction of phenolic compounds and promoting condensation between anthocyanins and tannins, allowing good colour stabilisation.

3.5 Microbiological Strategies

As the microbial strategy is an easy to implement and less expensive option, metabolic engineering of Saccharomyces cerevisiae for the production of reduced ethanol has been a very active field in the last two decades. The development of low-alcohol yeast raises several scientific issues. Firstly, a major challenge is to redirect grape must sugars towards the production of other by-products than ethanol while maintaining redox and energy homeostasis. Secondly, since the metabolic network is strongly interconnected, modification of central carbon metabolism often leads to large and sometimes unpredictable effects on the production of secondary metabolites. A critical issue in this matter is to maintain the performance of the yeast while avoiding the accumulation of metabolites that can have a detrimental effect on wine quality. Various approaches, mostly based on genetic engineering have been and for the first time, a wine yeast strain with reduced ethanol yield was generated (Tilloy et al, presented).

3.5.1 Genetic engineering

Two main genetic strategies have been developed: the first is the conversion of sugars into metabolites that cannot be fermented and are therefore no longer destined for ethanol production; the second is the modification of the central carbon pathway to divert it from ethanol production with the consequent accumulation of other end products. This has been achieved by targeted modification of specific metabolic pathways involved in redox metabolism (e.g. by increasing lactate or glycerol production) or by working directly on NADH to decrease the availability of this cofactor for alcohol dehydrogenase.

A first approach is based on the expression of lactate dehydrogenase (LDH) in S. cerevisiae (Dequin & Barre, 1994), in which, pyruvate is diverted to lactic acid formation at the expense of the alcohol production pathway. This acid is an interesting compound because of its lack of flavour and its acidifying properties. In our selected yeast, lactic acid plays the same role as ethanol as an electron acceptor, without affecting the oxidation-reduction balance. However, by following this route, a quantity of lactate, well above acceptable levels (> 10 g/L), is produced.

Another strategy is based on the expression of a bacterial NADH oxidase in yeast so that it competes with the fermentative alcohol dehydrogenase. Expression of this gene resulted in a 15% reduction in ethanol production, but with an increase in acetaldehyde production and stuck fermentation (Heux et al., 2006b). However, the most efficient technique was to divert the metabolism towards increased production of glycerol (Figure 1C). In S. cerevisiae, this compound plays important role in redox homeostasis and resistance to osmotic stress (Blomberg and Adler, 1992). This alcohol is thought to contribute to the smoothness and overall body of the wine and its production has led to a substantial decrease in ethanol (Michnick et al., 1997; Remize et al., 1999; de Barros Lopes et al., 2000) but the accumulation of compounds undesirable for sensory quality such as acetate and acetoin (Remize et al., 1999; Cambon et al., 2006). Genetic engineering at the acetaldehyde branch point has made it possible to limit the accumulation of these compounds, resulting in the development of low-alcohol strains with carbon flow redirected towards glycerol and 2,3-butanediol, which is without sensory impact in wines (Cambon et al., 2006; Ehsani et al., 2009). In this strain, the ethanol yield was reduced by 19%.

A further alternative approach is the selection of yeast variants using adaptive evolution. This involves cultivating yeast populations over a long period under selective conditions from which variants with higher suitability for the environment will then be selected but finding the selective conditions to drive metabolism towards a particular desired product remains challenging. In the research, the pentose phosphate (PP) pathway, an alternative route to glycolysis for sugar catabolism, was first targeted (Figure 2A). The diversion of carbons to this pathway was expected to have various

consequences on yeast metabolism, including reduced availability of carbons for ethanol production and reduced acetate production (the second pathway for NADPH production). Using this approach, we obtained evolved strains with a 1.5-fold increase in flux through the PP pathway. This increase had very little impact on ethanol production, but the evolved strains had several new properties, including reduced acetate production and significantly increased ester production (Cadière et al., 2011; Cadière et al, 2012). Adaptive evolution was also applied to increase glycerol production. A first approach was based on the use of alkaline pH sulphite as a selective agent (Kutyna et al., 2012) from which a variant producing 30% more glycerol was obtained and increased sulphite tolerance was achieved. However, this variant was not sufficient to substantially reduce ethanol production. Subsequently, the osmolyte property of glycerol which is mediated by HOG (high osmolarity glycerol) was tested using osmotic/saline stressors and evolved strains were obtained that had 167% more glycerol production and 6% less ethanol production, showing that adaptive evolution is a potentially viable alternative for engineering low-alcohol wine yeast strains.

Although such strategies cannot generate a carbon flux diversion as high as the one obtained with genetic engineering, the availability of such strains with good general attributes, capable of reducing the alcohol content of wine from 0.5 to 1 % vol offers exciting prospects.

3.5.2 Non-conventional yeasts

In recent years, unconventional yeasts such as *S. uvarum* species, interspecies hybrids of *S. cerevisiae* and non-Saccharomyces yeasts have been studied as an alternative for alcohol reduction in wine.

S. uvarum is a species characterised by its cryotolerance and its ability to produce high levels of 2-phenylethanol and its corresponding acetate. In contrast, at 24 °C, its low tolerance to ethanol was clearly revealed as a discriminating trait, distinguishing this species from *S. cerevisiae* (Masneuf-Pomarède et al., 2010; Naumov et al., 2002; Castellari et al., 1994; Rainieri et al., 1999; Walsh e Martin, 1977; Kishimoto e Goto, 1995). 66 yeast strains were used in this study: 7 *S. cerevisiae,* 4 *S. uvarum* and 55 synthetic hybrids (28 interspecific and 27 intraspecific). Fermentations were carried out in triplicate at both 18°C and 16°C in Sauvignon Blanc grape must containing 188 g/L of sugar. Several phenotypic traits were measured: fermentation kinetics, yeast population, aroma profiles and fermentation products. fermentation products. The analytical results obtained from fermentation at 26 °C showed variations but there was no significant difference in alcohol/sugar production between the three groups of strains (*S. cerevisiae, S. uvarum,* interspecific hybrids). On the contrary, the results obtained from fermentation at 18 °C were interesting because as shown in Figure 17, the *S. uvarum* group (parent and intraspecific hybrid strains), in particular hybrid EU23, produced 0.30 %vol less ethanol than the *S. cerevisiae* group, while intermediate ethanol production was observed for the

interspecific hybrid group with high performance for some strains. This interesting hybrid was then also tested in synthetics with higher sugar content and compared to 10 strains usually used for winemaking, again showing an average of 0.34 %vol. less ethanol was produced at 18°C than the 10 starter strains.

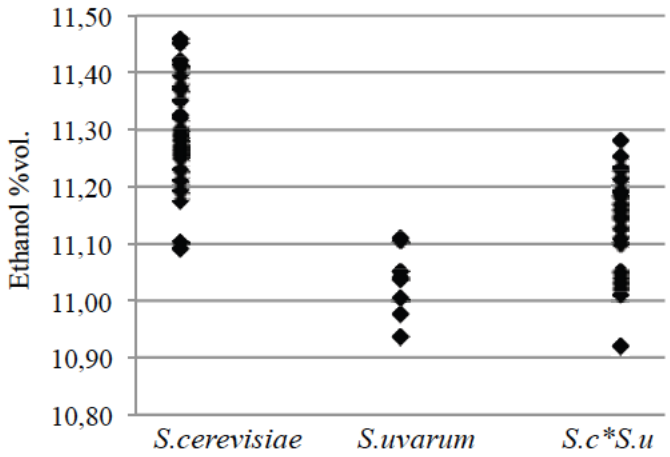

Figure 16. Ethanol production of S. cerevisiae, S. uvarum and interspecific hybrids at 18°C (Bely et al., 2013).

During fermentation, the predominant non-Saccharomyces yeasts belong to the *Hanseniaspora, Kloeckera, Pichia, Candida* and *Metschnikowia* genre but others may also be present, such as *Torulaspora delbrueckii, Schizosaccharomyces pombe, Kluyveromyces spp*. etc. These species start fermenting until the nutrients are depleted, the increase in ethanol content and the increase in heat will gradually eliminate the less tolerant species, thus favouring the development of *S. cerevisiae* which will complete the fermentation (Heard and Fleet, 1985).

Specifically, two species, *Torulaspora delbrueckii* and *Candida zemplinina* were evaluated in the laboratory and compared to the *S. cerevisiae* species. Regarding T.delbrueckii, fermentations were carried out in triplicate at 24 °C. The results confirmed the low volatile acidity and glycerol production of this yeast (Herraiz et al., 1990; Ciani and Maccarelli, 1997; Ciani and Picciotti, 1995; Moreno et al., 1991). Furthermore, this species is characterised, among the non-Saccharomyces, by a good production of ethanol. In fact, the majority of the strains used for experiments produced between 8 and 11% and 7 and 10 % ethanol vol. at 17 °C and 24 °C (Renault et al., 2009), however, no significant differences were observed between *T. delbrueckii* and *S. cerevisiae.*

Recent studies conducted over 5 years have suggested the possible use of *C. zemplinina* in winemaking. Evaluations carried out on some technological properties of different wine species have shown that this species is highly fructophilic, osmotolerant and a high producer of glycerol (Tofalo et al, 2012; Sadoudi et al., 2012; Sipiczki, 2003; Rantsiou et al., 2012). The follow-up study aimed to

investigate the potential application of this species in mixed starters with *S. cerevisiae.* Forty-eight strains of *C. zemplinina* were isolated, and three of *S. cerevisiae* were tested for their fermentation performance. All fermentations were carried out on Merlot must containing 240 g/L sugar at 24°C. As in the case of *Torulaspora*, all fermentations were carried out in triplicate. All strains of S. cerevisiae completed alcoholic fermentation, while inoculating musts with C. zemplinina showed fermentation arrest, confirming the low fermentation capacity. The results indicate significant differences between the yeast strains in terms of ethanol production, volatile acidity and glycerol (Table 23), indeed the *C. zemplinina* group has a low yield, about 12% less than the *S. cerevisiae* strains, which is partially explained by the production of glycerol.

	C. zemplinina (n=48)			*S.cerevisiae* (n=3)	
	Minimum	*Maximum*	*Average*	*Average*	*Average*
Ethanol (%vol.)	6.6 ± 0.1	9.9 ± 0.7	7.7 ± 0.8	7.94* ± 1.49	13.9 ± 0.3
Residual sugar (g/L)	48.3 ± 13.9	94.3 ± 1.9	79.1 ± 11.2	91.1 ± 21.5	1.3 ± 0.1
Yield ethanol/sugar (g/g)	0.35 ± 0.00	0.41 ± 0.00	0.37 ± 0.01	0.42 ± 0.01	0.46 ± 0.01
Volatile acidity (g/L acetic acid)	0.48 ± 0.01	1.15 ± 0.06	0.72 ± 0.17	0.18 ± 0.05	0.33 ± 0.02
Glycerol (g/L)	11.9 ± 0.8	14.8 ± 0.8	13.6 ± 0.7	6.09 ± 0.34	7.6 ± 0.3

Table 23. Main oenological characters in Merlot wine of pure cultures at 24 °C (Bely et al., 2013).

** Fermentations stopped before the end of fermentation*

Combined fermentation between the two was also analysed to reduce alcohol production. The *C. zemplinina* yeasts were classified into 2 groups according to olfactory perception, one negative due to the production of undesirable flavours (sulphur compounds) and another with neutral or acceptable flavours. Sequential fermentations were carried out inoculated with C. zemplinina (107 viable cells/mL), followed by S. cerevisiae (2.106 viable cells/mL) after 24 or 48 hours of fermentation, leading to reductions in ethanol production of 0.39 to 0.90 % (Table 24).

	Sequential cultures *C. zemplinina*/*S.cerevisiae*						Pure culture
	Strain 401		Strain 629	Strain 261	Strain 153	Strain 278	*S. cerevisiae*
	24h	48 h	48h	48h	48h	48h	FX10
Ethanol (%vol.)	13.16 ± 0.07	13.52 ± 0.18	13.46 ± 0.05	13.41 ± 0.08	13.51 ± 0.04	13.01 ±0.04	13.91 ± 0.00
Residual sugar (g/L)	0.87 ± 0.14	0.90 ± 0.20	0.90 ± 0.10	1.33 ± 0.75	0.83 ± 0.06	0.95 ± 0.07	0.95 ± 0.07
Yield ethanol/sugar (g/g)	0.43 ± 0.00	0.45 ± 0.00	0.44 ± 0.00	0.44 ± 0.00	0.45 ± 0.00	0.43 ± 0.00	0.46 ± 0.00
Volatile acidity (g/L acetic acid)	0.45 ± 0.01	0.84 ± 0.15	0.83 ± 0.07	0.76 ±0.04	0.81 ± 0.07	1.01 ± 0.06	0.31 ±0.04
Glycerol (g/L)	13.03 ± 0.87	14.72 ± 0.80	15.36 ± 0.95	15.76 ± 0.62	15.21 ± 0.46	15.76 ±0.01	7.30 ± 0.48

Table 24. Main oenological characters in Merlot wine of sequential cultures C.zemplinina/S.cerevisiae at 24 °C (Bely et al., 2013).

The use of *C. zemplinina* in sequential culture seems promising with regard to low ethanol production, but further study is still needed in order to propose strains of this yeast with neutral impact on the organoleptic perception of wine. Sequential cultures produced 1.8 to 2.2 times more glycerol than *S. cerevisiae* alone, confirming the glycerol production characteristic of this yeast. Considering the better yield in alcohol/sugar, glycerol, and volatile acidity, the best multi volatile acidity, the best sequential multistarter was *C. zemplinina* 401 with *S. cerevisiae* added after 24 hours of fermentation. after 24 hours of fermentation. The use of C. zemplinina in sequential culture seems promising concerning low ethanol production, but further study is still needed to propose strains of this yeast with a neutral impact on the organoleptic perception of wine.

4. OIV Rules

It is important for the wine sector to consider a response to market signals suggesting emerging consumer interest in products containing less alcohol. Therefore defining the following product categories is crucial in order to provide greater choice for wine consumers and market niches for wine business operators. As an intergovernmental organisation, the OIV has among its contributions to the international harmonisation of existing practices and standards and, if necessary, the development of new international standards to improve the conditions under which wine products are produced and marketed. Since 2004, OIV member states have taken the decision to obtain a partially dealcoholised wine by a process of reducing the ethanol in the wine to no more than 2% alc. vol.

For the definition of wine, there are different minimum actual alcoholic strengths in different countries and also according to product categories, but the OIV only sets a single minimum actual alcoholic strength 8.5% vol. for the European Union with a derogation to 4.5% vol. for certain geographical indications, recently Australia reduced the limit from 8% vol. to 4.5% vol. For Argentina and China, the limit is based on 7% vol. while for some other countries, there is no specified minimum. Within this framework, in 2012, the General Assembly adopted new resolutions in 2012 (OIV-ECO 432-2012 and OIV-ECO 433-2012) which include two new definitions: "beverage obtained by dealcoholisation of wine" and "beverage obtained by partial dealcoholisation of wine".

Resolutions OIV-OENO 394A-2012 and OIV-OENO 394B-2012, on the other hand, specify the separation techniques that can be used for dealcoholising wines or for correcting the alcohol content of wines, respectively.

On the other hand, through the adoption of the OIVOENO 394A-2012 and OIV-OENO 394B-2012, the separation techniques and the conditions for the reduction of the alcohol content of wine, distinguishing between a correction of the alcohol content and a de-alcoholisation of the wine.

With regard to the partial dealcoholisation of wine, a maximum reduction of 20% of the initial alcohol content and the products obtained through this practice must still comply with the definition of wine and above all maintain the minimum alcoholic strength of wine, if presented as such.

If the alcohol content of the wine is reduced by more than 20%, it will fall under a process of de-alcoholisation, which means removing part or almost all of the ethanol content in the wine in order to develop wine products with low or reduced alcohol content. This process is permitted, but the resulting product must not be presented as wine, as it will not comply with the definition of wine.

The separation techniques that can be used to achieve one of these objectives are partial vacuum evaporation, membrane techniques and distillation.

These procedures must not be used on wines with other organoleptic defects and the elimination of alcohol in the wine must not be done at the same time in conjunction with a modification of the sugar content in the corresponding musts.

In addition, these processes consist of separating the must or wine into several fractions with different chemical compositions that must comply with the objectives and OIV Resolutions Oeno 373A-2010 and Oeno 373B-2010.

Some examples of requirements are that the wine or must be treated must comply with the definitions and limits of the OIV, or that these techniques may not be used to cover up fraudulent acts. Untreated fractions or fractions treated with OIV approved practices must only be blended with must or wine fractions obtained by separation techniques from the same starting product with the only exception of fractions used as wine-based products as defined in the International Code of Oenological Practices. Another aspect is that recombination must take place in the shortest possible time and in the same place whenever possible. For the processing of products with reduced alcohol, it is also possible to reduce the sugar level of the must before fermentation. In this context, the OIV has adopted a resolution concerning the reduction of sugar content in must musts, which describes the objectives and requirements for achieving these objectives (OIV-OENO Resolution 450A-2012). In general, terms, reducing the sugar content of musts excludes dealcoholisation of the wines from which they are made and should not be used in combination with must and wine enrichment techniques. This process is limited due to the significant reduction in volume and results of the separation techniques used and the treatment must be carried out on a volume of must which is determined according to the required result in terms of sugar content reduction.

With the directives just shown, the member states of the OIV have updated the International Code of Oenological Practices by inserting two new product definitions: "beverage obtained by partial dealcoholisation of wine" for products with an alcohol content between the minimum required for wines and 0.5% v/v, and "beverage obtained by dealcoholisation of wine" for products with an alcohol content of less than 0.5%v/v.

5. Conclusions

This study aimed to illustrate in concrete terms the consequences of climate change and to analyse the various procedures for reducing the alcohol content of wine, which is one of the fundamental consequences of global warming.

The changes associated with climate change in grape quality will pose significant challenges for winemaking and final wine quality, particularly about the expression of varietal aromas, microbiological and chemical stability and sensory balance. Conditions in warm wine-growing regions, where future development is likely to have an overall negative impact on quality, have already been noted. Several wine-growing regions may become unsuitable for wine production for quality wine production in this century, while several regions may have to rethink current terroir concepts regarding cultivar selection and winemaking technology, in the case of European viticulture. Even in colder climates that are thought to have benefited from climate change Even in cooler climates that are said to have benefited from climate change, a more interventionist style of winemaking involving water additions, acid adjustments and alcohol reductions may be required in the future. In recent years, traditional wine-growing regions in the 'old world' and those of the 'new world' (Canada, USA, Chile, Argentina, South Africa, Australia, New Zealand, China) have been joined by new regions that have been termed 'new latitudes' and include sites with tropical climates such as Peru, Thailand, Cambodia, India, Brazil, and Venezuela (Possingham, 2008; Shaefer, 2008). The method chosen by wine producers to moderate ethanol levels is often determined by the consideration of the style of wine, the volume of production, the level of ethanol to be removed, the operational expenses, the flexibility for the use of equipment and staff training requirements. For many wine producers, it will be important to implement strategies that cover the entire wine production process, starting with the vineyard site and varietal selection, practical management practice, careful control of fermentation parameters together with the judicious use of processing technologies to produce wines with lower ethanol concentrations. However, no single approach is likely to produce a significant reduction in alcohol without a substantial alteration in the sensory properties of the product. Such alterations in the sensory properties of the product result from loss of aroma and alteration of properties such as body, warmth, sweetness, and perception of bitterness and acidity. The extent of the sensory change in wines reduced the presence or absence of specific classes of compounds responsible for the varietal aroma. Likely, the best treatment approach for the removal of ethanol from specific wines by membrane processes will be determined by the varietal composition and careful optimisation of operating conditions such as temperatures, flow rates, stripping, condensation and aroma recovery rates. Viticultural strategies to address this problem are all about reducing the sugar content of the grapes at harvest, as this is the starting point for obtaining less

alcohol in wine. These strategies are mostly based on the correct use of certain cultivation techniques that have a direct or indirect impact on the sugar content of the such as irrigation, pruning and canopy management, as well as the use of a variety of other techniques; however, they need to be revisited to achieve the desired result. The use of antiperspirants to spray on the leaves and growth regulators to apply on the grapes can be considered as useful tools, in general, all techniques that limit photosynthesis by changing the source-sink ratio or delaying fruit ripening can achieve the objective. However, this is not always easy to achieve since the vine has high physiological plasticity that provides different compensatory responses that act concerning imposed treatments. In addition, together with a low alcohol level, colour intensity and a rich aromatic and phenolic profile are also required parameters for quality wines, but grapes harvested at a total soluble solids concentration suitable for producing low alcohol wines may not have enough phenols, especially anthocyanins. Experimental results available at the moment show often unequivocal indications. This is a consequence of the fact that each viticultural technique can have different applications, and that the interaction between the vine, the rootstock and the environment are very complex. It has to be considered that most of the experimental results derived from research on vineyard management are not specifically dedicated to obtaining grapes for low-alcohol wines, so more studies on this topic should be done to clarify the issue. According to other examples presented, the exploration of the genetic capacity of different indigenous cultivars, clonal selection, as well as the exploitation of rootstock-splitting interactions can help to meet the new challenges. Another winemaking process that can be adopted is the use of unripe grapes, which can be useful for partially reducing the alcohol content and simultaneously lowering the pH of the wines. In addition, it was seen that the colour of wines with reduced alcohol content was better than their corresponding controls and their phenolic composition was similar. Considering also that the procedure proposed in this article requires no additional equipment and is easy to apply in standard cellars, it can be safely used for reducing the alcohol content of wine. The results regarding reverse osmosis indicate that the application to the partial dealcoholisation of wine does not appreciably alter the colour or chemical composition, with the sole and intended decrease in ethanol content. Furthermore, a panel of trained tasters as seen had serious difficulty distinguishing between control and partially dealcoholised wines in triangular trials. This technique, therefore, can be very useful for the partial dealcoholisation of red wine, also because the cost of the process can be considered affordable as the equipment manufacturer provides the service at 0.15 €/L for the removal of 1% ethanol. Reverse osmosis is, therefore, an interesting tool especially nowadays as climate change is increasingly causing a mismatch between pulp maturity and phenolic maturity of the grapes (Gil et al., 2013). Regarding the Pervaporation survey has shown that the working temperature plays the most important role in the production of low-alcohol and alcohol-

free wines. The results of the pervaporation survey show that the working temperature plays the most important role in the production of low-alcohol and alcohol-free wines, so the choice of this parameter is the main objective to optimise the process. At higher temperatures, the permeate flux is higher and the membrane surface requirement is lower, so it is economically advantageous because it is a faster process but at higher temperatures the membrane separation efficiency and separation capacity decrease. Therefore, to avoid a serious loss of aroma, the choice of lower pervaporation temperatures is the most favourable. Economic analyses predict a large investment cost, which can be explained by the relatively high price of non-porous membranes, but the investment could be remunerated in a few years. The use of nanofiltration for the removal of alcohol from wine has proved to be an effective technique. Its use has been shown to have certain advantages over reverse osmosis, such as a reduction in volatile acidity and a lower loss of anthocyanins while maintaining the aromatic profile as we have seen. In addition, its application also allows a more extensive removal of alcohol, enabling the production of low-alcohol and de-alcoholised wines. and de-alcoholised wine. Replacement of mature grape must by immature grape juice and pre-fermentative hot maceration are further technological alternatives to improve the colour of red wines, in this case concerning the Tannat variety. The effect of must replacement on the colour and general composition of wines depends very much on the composition of the starting grapes. In contrast, pre-fermentative hot maceration improved the intensity and quality of wine colour by increasing the extraction of phenolic compounds and promoting condensation between anthocyanins and tannins, suggesting greater colour stability. The results obtained from this research were interesting, as this technique showed us that it can limit the extractability of anthocyanins. Furthermore, this vinification technique modified the anthocyanin profile of wines in which a relative increase in the most oxidisable forms was obtained, but further studies should be carried out. Another major current challenge is to develop "low alcohol yeasts" using technologies that are acceptable to the consumer and that can be used by winegrowers. We have recently obtained evolutionarily engineered yeasts with sugars towards glycerol and 2,3-butanediol, making it the first example of a low alcohol yeast strain for the wine market capable of reducing the alcohol content of wine from 0.5 to 1 % vol/vol. Although indeed the reduced ethanol content can be considered low, these strains could be an essential tool in a strategy involving a combination of different approaches, such as vine varieties, physical and biological methods. There is a growing demand for wine products with reduced alcoholic strength and wine producers are very interested in this possible new offer. The OIV is working on this plan to harmonise the definition of low-alcohol wine and the practices for obtaining it, as precisely the lack of definitions, designations and practices could turn into barriers to trade by hampering innovation and the competitiveness of businesses in this sector.

Climate change is inevitable, we can only adapt to it and try to mitigate its effects. These techniques are now available and can be very useful to offset the effects of global warming in our wines.

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