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How to adapt winemaking practices to modified grape composition under climate change conditions

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Abstract

Aim: In the context of climate change, adaptation of enological practices and implementation of novel techniques are major challenges for winemakers. The potential interventions are linked in particular with the alcohol content and the global acidity of wine. Here, we review current microbiological and technological strategies to overcome such issues.

Methods and results: Reducing ethanol concentration poses a number of technical and scientific challenges, in particular looking for specific yeast strains with lower alcohol yield. Several non-genetically modified organism (GMO) strains – *S. cerevisiae* or interspecific hybrids of the *Saccharomyces* genus – have yet been developed using different strategies, and some of them allow decreasing the final ethanol concentration by up to 1%. Several membrane-based technologies have also been developed not only to reduce the ethanol content of wines but also to increase the acidity and more generally to control the wine pH.

New strategies are also proposed to improve the control of winemaking, especially the management of alcoholic fermentation of sugar-rich musts and the control of oxidation during the process.

Conclusion: Reducing ethanol of wines and increasing their acidity are good examples of novel techniques of interest in the context of climate change. Other strategies are still under study to adapt winemaking practices to changes in grape composition.

Significance and impact of the study: Membrane-based technologies can be used to reduce the ethanol content of wines or to increase the acidity. Microbiological strategies will also be soon available for winemakers.

Keywords: Climate change, winemaking, yeast, alcohol, acidity

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Introduction

The major expected effects of climate change are an increase in temperature and changes in rainfall patterns and incoming radiation. As a consequence, vine phenology and grape composition at harvest should be dramatically modified. The main consequences on grapes are more sugar and less organic acids; aromas and phenolic compounds of wines will also be affected.

Excessive alcohol in wines exerts a number of negative effects that raise microbiological, technical, sensory and economic challenges. First, because ethanol is a chemical stress factor for yeast that is often the underlying cause of sluggish or stuck fermentation. Second, because excessive ethanol levels also impair the sensory quality of the wine by increasing the perception of hotness and by altering the perception of wine aroma complexity (Goldner *et al.*, 2009). Today, wines with moderate ethanol levels are often preferred, in accordance with health prevention policies.

Consequently, reducing alcohol levels in wine has inspired considerable efforts in the past decade and remains a major challenge for the coming years. Different approaches to reduce alcohol levels in wines have been proposed at all stages of the winemaking process.

Some viticultural strategies are promising. Thanks to classical breeding research programs, different new grape varieties were created by successively crossing *Muscadinia rotundifolia* and 4 different *Vitis vinifera* cultivars. Such varieties reach full maturity with lower sugar content (i.e. 180 g/l) (Aguera *et al.*, 2010). Two of these new varieties are now being tested in the experimental vineyard of Inra Pech-Rouge at a 1-ha scale. Another advantage of these new grape varieties is their natural resistance against powdery and downy mildew, thus preserving environment from pesticide use.

In a shorter-term perspective, several physical techniques for dealcoholization have been developed (Schmidtke *et al.*, 2012). Another prospect is the development of yeasts with reduced alcohol yield, which is both very promising and challenging (Tilloy *et al.*, 2015).

High pHs are more and more common, in particular in regions located at low latitudes, and acidification may become inevitable for the process (effectiveness of SO_2, avoidance of oxidation, etc) and with regard to organoleptic properties. Addition of tartaric acid is often used but with inconsistent results. Membrane-based technologies have been developed to decrease the pH of wines by removing potassium content (Lutin *et al.*, 2010) and more generally to control wine pH. Besides these new technologies, adapting winemaking practices may be an effective way to counteract the effects of changes in grape composition. This is particularly the case for fermentation management and oxidation control.

Microbiological strategies to reduce ethanol content of wines

1. Reducing ethanol yield of *Saccharomyces cerevisiae*

The yeast *S. cerevisiae* has been the subject of intensive research for metabolic engineering. Strong fundamental knowledge of its genetics, physiology, systems biology and genomics has facilitated its use as a metabolic engineering platform (Nevoigt, 2008; Borodina and Nielsen, 2014). These approaches have been widely used to optimize various traits of interest in the field of food and fermented beverages. In the recent years, reducing ethanol yield has been one of the major targets.

The rate of conversion of sugars to ethanol presents only minor variations between strains of *S. cerevisiae*. Hence, to decrease the ethanol production in this yeast, it is necessary to reroute the flux of carbons to other pathways and to the production of other secondary metabolites. However, there are several constraints: it is essential to maintain the redox balance, to avoid the production of compounds that could affect the organoleptic quality of the wines, and to preserve yeast performance.

To achieve this goal, numerous metabolic engineering strategies have been implemented, such as the expression of a NADH oxidase and a NADPH-dependent lactate dehydrogenase, and the overexpression of *GPD1* (reviewed in Tilloy *et al.*, 2015). Among those various strategies, rerouting carbons towards glycerol has emerged as the best option to reduce ethanol yield (Michnick *et al.*, 1997; Remize *et al.*, 1999; Cambon *et al.*, 2006; Varela *et al.*, 2012).

In *S. cerevisiae*, glycerol plays major roles in redox homeostasis and osmotic stress resistance as it is the main compatible solute in yeast (Blomberg and Adler, 1989). Glycerol is usually found in wines at concentrations ranging from 5 to 9 g/l.

As a proof of concept, wine yeast strains overproducing glycerol and 2,3-butanediol, a polyol with no sensory impact on wines, with a lower

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ethanol yield and without accumulation of unwanted byproducts have been successfully constructed by metabolic engineering strategies (Ehsani *et al.*, 2009). These strains have the potential to decrease alcohol levels in wines by up to 3% (vol/vol). In recent years, due to the poor public acceptance of genetically modified (GM) strategies, alternative strategies such as evolutionary engineering have been favored.

Evolutionary engineering has proven to be successful to reshape yeast metabolism (Figure 1). The concept of adaptive evolution is that microorganisms tend to evolve their intrinsic characteristics to adapt to new conditions. During this process of evolution, random genetic mutations occur, and if a selection pressure is applied, strains having one or several beneficial mutations in the selective medium will dominate in the culture medium and can thus be selected. This approach is based on the extended cultivation of a strain in controlled selective medium to select for natural genetic variants having beneficial mutations under the conditions used. Since the emergence of mutations is a rare event, several hundred generations are usually necessary before observing an evolution, which can last several months.

Adaptive evolution of a commercial wine yeast strain (Lalvin EC1118®) has been carried out to divert yeast metabolism towards increased glycerol and lower ethanol production using hyperosmotic stress as selective condition. Serial transfers were performed during 300 or 450 generations using either sorbitol or KCl as osmostress or salt stress agent.

The approach with KCl stress succeeded in generating strains with redirection of carbon flux towards glycerol. After 200 generations under KCl stress, a first adaptation was detected and the observed increase in glycerol production was maintained after 250 and 300 generations.

The evolved strains showed a gain in fitness conferred by a better viability under salt stress and carbon starvation conditions, which was correlated to a greater glycerol production.

Detailed characterization of the KCl-evolved mutants during wine fermentation showed that the evolved strains have substantial changes in central carbon metabolism. Carbons are rerouted towards glycerol, succinate and 2-3-butanediol at the expense of ethanol and without the accumulation of undesirable compounds such as acetaldehyde, acetate and acetoin.

The concentration of the most abundant byproducts after 30 days of fermentation was determined (fermentation was conducted on a synthetic medium containing 260 g/l glucose and 210 mg/l of assimilable nitrogen, as described by Bely *et al.*, 1990). Carbon and redox balances were close to 100% for all strains. All evolved strains produced glycerol at concentrations 48 to 67% higher than that produced by Lalvin EC1118®, and the ethanol content in the synthetic wines was reduced by 0.45 to 0.80% (vol/vol). The evolved strains also produced larger amounts of succinate and 2,3-butanediol. In contrast, unlike previously described with engineered strains, no significant changes in the production of acetate and acetoin by the evolved strains were found, and the production of acetaldehyde remained in the range of the level found in wines. The evolved strains exhibited an overall decrease in fermentation performance in comparison to the ancestral strain but were nevertheless able to complete the fermentation. The final cell populations were the same between the ancestral strain and these two evolved strains.

To understand the metabolic changes underlying these phenotypic differences, the transcriptome and the endo-metabolome of the evolved mutants and of the ancestral strain were compared. The mechanisms underlying the overproduction of glycerol in the evolved mutants remain intriguing. Indeed, yeast facing severe KCl stress could have evolved the structural or regulatory genes that are related to glycerol metabolism. However, no significant changes in the transcription levels of genes involved in glycerol metabolism or in the high osmolarity glycerol (HOG) pathway and its target genes that would explain the increase in glycerol production were found. This suggests that glycerol overproduction is not mediated via increased protein

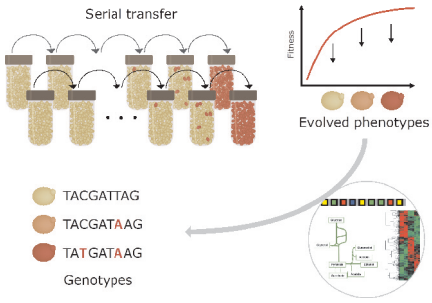

Figure 1. Evolutionary strategy by serial batch cultivation.
Prolonged cultivation is used to enable selection for successive mutations that confer a selective advantage (as higher biomass, growth rate or survival). Evolved phenotypes are analyzed by genome sequencing and a combination of omics to identify the underlying mutations. The mutations can be reconstructed in the ancestral background to validate their impact on the evolved phenotype (Tilloy *et al.*, 2015).

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synthesis of the glycerol pathway enzymes. Mutations in the evolved strains affecting post-transcriptional mechanisms could also modulate the reaction rates of glycerol production. The increased glycerol production could also be due to a global metabolic effect resulting from metabolic flux changes in central carbon metabolism.

The exact nature of the factors that caused the metabolic rearrangements in the evolved strains during the evolution experiment is difficult to identify. Whole genome sequencing revealed many changes between the ancestral and the evolved strains. Among these, a loss of heterozygosity (LOH) was observed in the evolved strains, particularly those selected in the late phase of the evolutionary process. Furthermore, in addition to the study of single-nucleotide polymorphisms (SNPs), quantitative trait locus (QTL) approaches were implemented to identify the molecular basis of this new phenotype. Bulk segregant analysis (BSA) was designed confronting a pool of segregants from an evolved high glycerol-producing strain to a pool of segregants derived from the parental strain. Candidate genes were identified and are currently under functional validation to confirm their implication in the phenotype.

To further increase the ability of the evolved strain to produce glycerol and to decrease its ability to produce ethanol during alcoholic fermentation, this strain was further subjected to conventional breeding. To this end, about 150 haploid yeast spores from the evolved strain K300.1(b) were generated and haploid strains of opposite mating type having the highest capacity to produce glycerol were selected. Mating between two spores producing 20.9 and 16.2 g/l glycerol during fermentation on MS medium generated a first generation hybrid. After sporulation and spore mating, a second generation hybrid, H2, which produced 16.8 g/l glycerol, was obtained.

To validate the results obtained at laboratory scale, we compared the behaviors and metabolic properties of K300.1(b), H2 and Lalvin EC1118® during pilot-scale fermentation using a Syrah grape must, at 28°C. To avoid stuck fermentation, oxygen and nitrogen were added during fermentation. H2 had a fermentation rate slightly lower than those of Lalvin EC1118® and K300.1(b), but all strains were able to complete the fermentation, despite the high sugar concentration (255 g/l). K300.1(b) and H2 produced more glycerol (14.1 g/l and 17.9 g/l versus 10.8 g/l) and slightly more succinate than Lalvin EC1118®. The ethanol contents of the wines produced by K300.1(b) and H2 were reduced by 0.6% (vol/vol) and 1.3% (vol/vol), respectively. The production of acetic acid by the evolved and hybrid strains was greatly reduced compared to that of Lalvin EC1118®.

The selected final strain was assessed at pilot scale on different grape musts during the 2013 and 2014 harvests. The wines obtained contained between 0.6 and 1% (vol/vol) less alcohol and very little volatile acidity. By contrast, their total acidity was consistently higher, which has a strong interest since the increase in wine alcohol content is most often associated with high pH (Tilloy *et al.*, 2015).

In summary, the results obtained in grape must at pilot scale confirm the metabolic shift of the evolved strain and show a greater metabolic reprograming of the hybrid derived from the evolved strain.

This study demonstrates that a combination of adaptive evolution and breeding strategies is a valuable alternative to rational engineering for the generation of non-GM, low ethanol-producing yeasts.

2. Reducing ethanol yield by using hybrids and non-*Saccharomyces* yeast

Interestingly, other species belonging to the *Saccharomyces* group (*S. uvarum* and *S. kudriavzevii*) differ in the metabolism for glycerol production and transport, NADH balance, and acetic acid production (Masneuf-Pomarède *et al.*, 2010; López-Malo *et al.*, 2013; Pérez-Torrado *et al.*, 2016). These species can be used as an original genetic material to get non-GM strains showing a partial reduction in ethanol content. However, their lower ethanol tolerance was clearly a discriminative trait, distinguishing these species from *S. cerevisiae*. As industrial winemaking may reach final ethanol content higher than 15° TAV, the low ethanol-producing strains must also have a strong ethanol tolerance. The benefits of hybridization have been described in a large range of plant and animal species in an agronomical context. One of the most striking consequences of hybridization is the phenotypic superiority of hybrids over their two progenitors (i.e. heterosis) (Lippman and Zamir, 2007). Hybridization may also confer an improved phenotypic stability over environmental change (i.e. homeostasis) (da Silva *et al.*, 2015).

Hybrids

In the *Saccharomyces sensu stricto* clade, hybrids can be easily obtained both at the intra or interspecific level allowing yeast improvement. Recently, we obtained a collection of 4 *S. uvarum* and 7 *S. cerevisiae* parental strains and all their 55 possible hybrids. The parental strains came from different

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beverage industries (wine, distillery and cider) as well as from nature isolates.

Fermentations were carried out at 2 temperatures (18°C and 26°C) in Sauvignon banc grape must containing 188 g/l sugar. The phenotypic distribution of 35 traits, i.e. fermentation kinetics, yeast population, aromatic profiles and fermentation products, was measured for the entire data set in triplicates (da Silva *et al.*, 2015). The ethanol/sugar yield (g ethanol/g of sugar consumed) was computed for the strains that achieved alcoholic fermentation leaving less than 1.6 g/l of residual sugar. Within strains of the same group, this parameter showed very low variability (CV <1.2%), underlining the strong robustness of this trait. Nevertheless, at 18°C we observed a significant reduction of ethanol production for the *S. uvarum* group with an average of 0.30% (vol/vol) reduction compared to the *S. cerevisiae* group (parental and intraspecific hybrid strains). The interspecific group showed an intermediate ethanol/sugar yield with some individuals producing 0.30% (vol/vol) less ethanol than the *S. cerevisiae* group mean. Interestingly, these interspecific hybrids have higher fermentation performances than the *S. uvarum* group due to the positive contribution of the *S. cerevisiae* genome. Within those hybrids, we selected one background of particular interest, EU23.

This hybrid was further tested in synthetic medium with higher sugar content (230 g/l) and compared to 8 strains of commercialized starters. At 18°C, EU23 showed a 0.34% (vol/vol) ethanol decrease related to glycerol overproduction (9.0 g/l compared to 6.5 g/l for the commercial strain group). As previously observed in Sauvignon blanc, the temperature slightly impacts the ethanol/sugar yield and only a difference of 0.15% (vol/vol) ethanol was observed in the same media at 28°C. In different red grape musts (Merlot and Cabernet-Sauvignon) this level of discrepancy (ranging between 0.15 and 0.32) was also observed at laboratory and at small pilot scale (30 l).

These results confirmed the interest of interspecific hybrids for lowering alcohol production. However, the reduction level obtained is not yet sufficient to guaranty a relevant result for an industrial application. Adaptive evolution experiments are now carried out by applying different osmotic stresses to this interspecific hybrid.

Non-*Saccharomyces* yeast

Several non-*Saccharomyces* yeast, such as *Torulaspora delbrueckii*, *Schizosaccharomyces pombe*, *Kluyveromyces* spp., *Issatchenkia* spp., *Zygosaccharomyces bailii*, etc, may also contribute to wine fermentation.

One species, *Candida zemplinina*, was evaluated and compared to *S. cerevisiae*. Forty-eight *C. zemplinina* isolates (mainly from Bordeaux must and also from Hungary and Italy must) were used. Three *S. cerevisiae* were also tested for their fermentation performance. Fermentations were carried out in pasteurized Merlot grape must containing 240 g/l sugar at 4°C.

In pure cultures, *C. zemplinina* yeast resulted in stuck fermentations, confirming the low fermentation capacity of this species reported in the literature. The *C. zemplinina* group possessed a low alcohol/sugar yield, 12% less compared to *S. cerevisiae* strains, which can be partially explained by the overproduction of glycerol.

We investigated the possibility of using *C. zemplinina*, as a partner of *S. cerevisiae*, in mixed fermentations by inoculating 10^7 viable cells/ml *C. zemplinina* with 2.10^6 viable cells/ml *S. cerevisiae*. In addition, sequential fermentations were inoculated

Table 1. Sequential cultures of *C. zemplinina*/*S. cerevisiae*.
Musts were inoculated with *C. zemplinina* (10^7 viable cells/ml), followed by *S. cerevisiae* (2.10^6 viable cells/ml) after 24 or 48 hours of fermentation. Means of triplicate fermentations ± SD. *: Significantly different from pure *S. cerevisiae* culture.

	Sequential cultures *C. zemplinina*/*S. cerevisiae*						Pure culture
	Strain 401 Seq 401		Strain 629	Strain 261	Strain 153	Strain 278	*S. cerevisiae*
	24h	48 h	48h	48h	48h	48h	FX10
Ethanol (%)	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
Res. 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 (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
Vol. acidity (g/l)	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

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with *C. zemplinina* (10^7 viable cells/ml), followed by *S. cerevisiae* (2.10^6 viable cells/ml) after 24 or 48 hours of fermentation.

All mixed cultures were able to complete fermentation. In our conditions, no significant differences in ethanol production were observed between *S. cerevisiae* FX10 Zymaflore culture and co-cultures (simultaneous inoculations) (data no shown). In contrast, sequential fermentations resulted in reductions of ethanol production from 0.39 to 0.90% (vol/vol) (Table 1). Note that for the sequential culture with *C. zemplinina* 401 a higher reduction is observed when *S. cerevisiae* is added earlier during fermentation. The best sequential multistarter was *C. zemplinina* 401 with *S. cerevisiae* added after 24 hours of fermentation.

Subsequently, a sensory evaluation was performed. Mixed culture wines were judged to be significantly different from those made by single *S. cerevisiae* culture. The latter scored better, while those made by the mixed cultures showed high sulfur off-flavor.

Further investigations are required to clarify the molecular mechanism underlying the reduction of ethanol production in *C. zemplinina* species. Its use in sequential culture appears promising regarding low-ethanol production, but an important effort has to be done to propose *C. zemplinina* strains with neutral impact on the organoleptic perception of wine.

Technological strategies

Microbial strategies still require additional studies to be fully effective when several new techniques and winemaking practices are – or will soon be – available to help the winemakers to correct the physico-chemical and sensory balances of wines.

1. Membrane-based technologies

Non-porous membrane technologies, due to their potential control by on-line sensors and their narrow specificity, provide powerful tools for the tuning of alcohol content and pH.

Reducing wine alcohol content

The removal of ethanol from wine after fermentation can be achieved by using various technologies like membrane filtration, distillation under vacuum or atmospheric pressure, spinning cone columns, adsorption (on resins, silica gels or zeolite), freeze concentration, evaporation and extraction using organic solvent or supercritical solvent. Among all these methodologies, semi-permeable membranes by which alcohol can be separated from wine have been applied for several years (Aguera *et al.*, 2010). Among membrane filtration techniques, reverse osmosis (RO) is the most used technique as it works at low temperatures with few negative effects on wine sensory attributes. Since water is removed along with alcohol, it should be added back to the treated wine or added to wine before RO application. Since addition of water to wine is prohibited in many countries, the resulting permeate should be coupled to steam distillation or membrane contactor technologies to separate water from ethanol (Bes *et al.*, 2010). However, regardless of the method used, the extent of aroma loss and change in flavor components increases with increasing amount of ethanol removed (Aguera *et al.*, 2010). Therefore, the sensory properties and acceptability of ethanol-removed wines may differ based on the final ethanol concentration and consumer preferences.

Adjusting wine pH

Tartrate stabilization by electrodialysis is the original «control and monitoring» electro-process, since it combines a mathematical model to calculate and predict the conductivity reduction needed on any wine to guarantee tartrate stability at -4°C/24.8°F for 6 days. Based on the same device, but using different membrane stacks, on-line acidification or de-acidification by bipolar electrodialysis system under enological conditions removes either potassium or organic acids from grape must or wine (Escudier and Le Gratiet, 2012). Depending on membrane type and process selected, it is possible to achieve either (1) the combined extraction of anions and cations to exactly reach the desired level of tartrate stability, (2) the exclusive extraction of cations to accurately adjust wine pH by a desired pH reduction, or (3) the exclusive extraction of anions to reduce wine acidity and increase the pH.

To decrease potassium content and consequently pH, an electro-membrane process with bipolar membranes has been successfully tested in wine (Lutin *et al.*, 2010). The influence of these processes on taste perception (sourness, bitterness, astringency) was investigated. Decreasing wine pH by 0.4 points using an electro-membrane technique significantly reduced the bitterness and enhanced the acidity of red wines, while no effect on astringency was observed (Samson *et al.*, 2009; Caillé *et al.*, 2011). Tannin composition was not modified by the electro-membrane treatment. 3.5 pH wines presented lower levels of monomeric anthocyanins but showed higher color intensity than 3.9 pH wines. These changes reflect a higher rate of conversion of monomeric anthocyanins to derived pigments at the lower pH

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Table 2. Comparative data of different treatments for dealcoholization of a wine from 14% (vol/vol) to 12% (vol/vol).
IO: Inverse Osmosis, D: Distillation, NF: Nanofiltration, MC: Membrane Contactor.
Italicized values = values estimated by calculation (Bes *et al*., 2010).

	IO-D	NF-D	IO-MC	NF-MC
Volume of permeate to be produced per liter of wine (%)	~ 25	~ 18	~ *50*	~ 30
Volume of water needed for the treatment (l water / l wine)	0	0	0.45	0.3
Nature of the co-product (effluent)	Alcohol ≥ 92% (vol/vol)		Alcoholized water (IO: ~ 4% (vol/vol), NF: ~7% (vol/vol))	

value. Whether these reactions are related to changes in taste properties (especially reduced bitterness of the lower pH wine) remains to be investigated (Müller *et al.*, 2007).

Bipolar membrane electrodialysis as well as use of anionic resins reduce the mineral content of musts, in particular NH_4^+ (Bouissou *et al.*, 2014). Therefore, acidification of musts that exhibit a low content of assimilable nitrogen may require nitrogen supplementation to avoid slow fermentations.

These new processes recently obtained OIV approval and are now authorized in Europe (reg. UE N°53/2011).

2. Control of key winemaking operations

Fermentation

One of the main concerns for enologists during winemaking is to ensure steady and complete fermentation so all the sugars in the must are converted to alcohol. When sugar concentrations are up to 250 g/l, achievement of complete fermentation is challenging and necessitates an optimal management of yeast nutrients.

Combining addition of ammoniacal nitrogen and oxygen is very efficient because of the positive effects of oxygen on yeast survival and of nitrogen on yeast activity (Sablayrolles *et al.*, 1996). However, this addition has to be correctly timed, i.e. at the start of the stationary phase, when about 5% ethanol has been produced (Blateyron and Sablayrolles, 2001).

Casalta *et al.* (2013, 2016) highlighted the interaction between grape solids (the main source of lipids) and assimilable nitrogen content, pointing out the importance of taking into account the balance between assimilable nitrogen and lipids for controlling fermentations during white winemaking. Thus, the strategy to control the alcoholic fermentation should not only take into account the addition of oxygen and nitrogen but also the lipid status of the must.

Soubeyrand *et al.* (2005) showed the advantage of adding protective nutritional elements during the rehydration phase. The yeast protector releases specific micronutrients and micro-protectors that move to the active yeast enhancing its effectiveness during alcoholic fermentation (Salmon and Ortiz-Julien, 2007). The micronutrients (vitamins and minerals) allow the yeast cells to reactivate their internal metabolism. The micro-protectors (specific sterols and polyunsaturated fatty acids [PUFA]) gradually integrate into the yeast cell membrane (Luparia *et al.*, 2004), strengthening it and facilitating exchanges with the exterior, thereby preventing the loss of the cellular material.

Oxidation

After the break of grape berry cell compartmentation during the various technological operations (de-stemming, pressing, crushing, etc), the dissolution of oxygen in the must leads to several oxidation reactions which modify, to varying degrees, the initial chemical composition of the must. This cellular disintegration brings together the substrates for oxidation – the native phenolic compounds of the grape –, oxygen and the enzymatic polyphenoloxidase activity (PPO) of the grape. The color of the must then changes to more of less pronounced brown tones with frequent changes in transparency (Cheynier *et al.*, 2010) and aroma (Hoffman *et al.*, 1996).

In the current context of decreased SO_2 effectiveness (reduction of the doses combined with pH increase of the musts), the behavior of the grape PPO activity during the winemaking process was recently studied (Frissant *et al.*, 2012; Sire *et al.*, 2016). This work led to new information on protection against oxidation during pressing, i.e. (1) the total inerting of a pressing system such as developed by certain manufacturers can be advantageously replaced by a reduction in the temperature of the grape harvest and its preservation

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during pressing and (2) the quality increase of the end juices of pressing can also be obtained by specific cooling of the grapes with a very strong sensory gain.

The use of inactivated yeasts enriched in glutathione during alcoholic fermentation was also studied in order to protect rosé and white wines from oxidation during wine aging (Aguera *et al.*, 2012). Aging of the corresponding wines in accelerated oxidative conditions or in standardized conditions clearly evidenced a protection of such treated wines against oxidation, from both a sensory and a chromatic point of view. This effect was particularly emphasized for the conservation of odorant varietal thiols.

In the last several years, it has been demonstrated that yeast cells, even in a non-viable physiological state, could exhibit a superfluous oxygen consumption capability, related to a mild oxidation of cellular lipids (Salmon, 2006). Such a potential of oxygen consumption by non-viable yeast cells was therefore applied for the protection of wines against oxidation during storage and/or aging. Yeast derivatives were therefore applied at enological doses on white wines stored under different conditions (with and without SO_2 protection). The obtained data suggested that such specific selected inactivated yeast could represent an innovative solution for the protection of wines against oxidation during storage and aging when compared to SO_2 addition in the protection against oxidative evolution of stored wines (Sieczkowski *et al.*, 2016a, 2016b).

Conclusion

Many different strategies can be used to adapt winemaking to consequences of climate change on grape composition. Some of them are already functional while others are still under investigation. The objective is not only to correct 'defaults' such as high ethanol concentrations or low acidity, but also to look for optimal strategies integrating viticultural and winemaking approaches to optimize wine quality.

References

Aguera E., Athès-Dutour V., Bes M., Caillé S., Cottereau P., Escudier J.L., Mikolajczak M., Roy A., Sablayrolles J.M., Samson A., Souchon I., Vidal J.P., 2010. Reduction of wine alcohol content: a comparative study of different technologies. *Bull. OIV* 83, 31-42.

Aguera E., Samson A., Caillé S., Julien-Ortiz A., Sieczkowski N., Salmon J.M., 2012. Apport de levures inactivées riches en glutathion en cours de fermentation alcoolique : un nouvel outil pour la protection des vins blancs et rosés contre l'oxydation. *Rev. Fr. Œnol.* 250, 2-11.

Bely M., Sablayrolles J.M., Barre P., 1990. Automatic detection of assimilable nitrogen deficiencies during alcoholic fermentation in oenological conditions. *J. Ferment. Bioeng.* 70, 246-252.

Bes M., Aguera E., Athès V., Cadière A., Cottereau P., Dequin S., Mikolajczak M., Roy A., Sablayrolles J.M., Souchon I., Samson A., Escudier J.L., 2010. Les différentes stratégies microbiologiques et technologiques de production de vin à teneur réduite en alcool. *Rev. Œnol. Tech. Vitivinic. Œnol*. 135, 9-10.

Blateyron L., Sablayrolles J.M., 2001. Stuck and slow fermentations in enology: statistical study of causes and effectiveness of combined additions of oxygen and diammonium phosphate. *J. Biosci. Bioeng.* 91, 184-189.

Blomberg A., Adler L., 1989. Roles of glycerol and glycerol-3-phosphate dehydrogenase (NAD+) in acquired osmotolerance of *Saccharomyces cerevisiae*. *J. Bacteriol.* 171, 1087-1092.

Borodina I., Nielsen J., 2014. Advances in metabolic engineering of yeast *Saccharomyces cerevisiae* for production of chemicals. *Biotechnol.* J. 9, 609-620.

Bouissou D., Escudier J.L., Salmon J.M., Casalta E., 2014. Étude de l'impact de l'acidification par électrodialyse à membranes bipolaires et par résines échangeuses de cations sur les composés azotés et les minéraux du moût de raisin. *Bull. OIV* 87, 537-556.

Caillé S., Picou E., Bouissou D., Escudier J.L., Samson A., 2011. Éude organoleptique de l'acidification de vins rouges de presse par ajout d'acide tartrique ou par procédé électromembranaire. *In: Actes de colloques du 9e Symposium International d'œnologie de Bordeaux*, pp. 1043-1047.

Cambon B., Monteil V., Remize F., Camarasa C., Dequin S., 2006. Effects of GPD1 overexpression in *Saccharomyces cerevisiae* commercial wine yeast strains lacking ALD6 genes. *Appl. Environ. Microbiol*. **72**, 4688-4694.

Casalta E., Cervi M.F., Salmon J.M., Sablayrolles J.M., 2013. White wine fermentation: interaction of assimilable nitrogen and grape solids. *Aust. J. Grape Wine Res*. **19**, 47-52.

Casalta E., Vernhet A., Sablayrolles J.M., Tesniere C., Salmon J.M., 2016. Review: Characterization and role of grape solids during alcoholic fermentation under enological conditions. *Am. J. Enol. Vitic*. **67**, 133-138.

Cheynier V., Schneider R., Salmon J.M., Fulcrand H., 2010. Chemistry of wine. In: *Comprehensive Natural Products II. Chemistry and Biology, Volume 3: Development and Modification of Bioactivity*, pp. 1119-1172. L. Mander and H.W. Lui (eds), Oxford, UK, Elsevier.

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da Silva T., Albertin W., Dillmann C., Bely M., la Guerche S., Giraud C., Huet S., Sicard D., Masneuf-Pomarede I., de Vienne D., Marullo P., 2015. Hybridization within *Saccharomyces* genus results in homoeostasis and phenotypic novelty in winemaking conditions. *PLoS One* **10**, e0123834.

Ehsani M., Fernández M.R., Biosca J.A., Julien A., Dequin S., 2009. Engineering of 2,3-butanediol dehydrogenase to reduce acetoin formation by glycerol-overproducing, low-alcohol *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol*. **75**, 3196-3205.

Escudier J.L., Le Gratiet Y., 2012. Precision winemaking for wine stability: continuous and adjustable control of tartrate stability and wine acidity without the use of oenological additives. American Society of Enology meeting, Portland, USA.

Frissant S., Delmas C., Souquet J.M., Samson A., Moutounet M., Salmon J.M., 2012. Management de la température de la vendange depuis la vigne jusqu'à la mise en fermentation : étude quantitative de la protection obtenue contre l'oxydation. *Rev. Œnol.* 145, 21-25.

Goldner M.C., Zamora M.C., Di Leo Lira P., Gianninoto H., Bandoni A., 2009. Effect of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine. *J. Sens. Stud.* 24, 243-257.

Hofmann T., Schieberle P., Grosch W., 1996. Model studies on the oxidative stability of odor-active thiols occurring in food flavors. *J. Agric. Food Chem*. 44, 251-255.

Lippman Z.B., Zamir D., 2007. Heterosis: revisiting the magic. *Trends Genet*. 23, 60-66.

López-Malo M., Querol A., Guillamon J.M., 2013. Metabolomic comparison of *Saccharomyces cerevisiae* and the cryotolerant species *S. bayanus* var. *uvarum* and *S. kudriavzevii* during wine fermentation at low temperature. *PLoS One* 8, e60135.

Luparia V., Soubeyrand V., Berges T., Julien A., Salmon J.M., 2004. Assimilation of grape phytosterols by *Saccharomyces cerevisiae* and their impact on enological fermentations. *Appl. Microbiol. Biotechnol.* 65, 25-32.

Lutin F., Bonneaud D., Le-Gratiet Y., Moutounet M., Bouissou D., Escudier J.L., 2010. L'acidification par électrodialyse bipolaire : une technique soustractive précise et performante, comme alternative au tartricage des vins. *Rev. Œnol. Tech. Vitivinic. Œnol*. 137, 24-25.

Masneuf-Pomarède I., Bely M., Marullo P., Lonvaud-Funel A., Dubourdieu D., 2010. Reassessment of phenotypic traits for *Saccharomyces bayanus* var. *uvarum* wine yeast strains. *Int. J. Food Microbiol*. 139, 79-86.

Michnick S., Roustan J.L., Remize F., Barre P., Dequin S., 1997. Modulation of glycerol and ethanol yields during alcoholic fermentation in *Saccharomyces cerevisiae* strains overexpressed or disrupted for GPD1 encoding glycerol 3-phosphate dehydrogenase. *Yeast* 13, 783-793.

Müller K., Picou E., Souquet J.M., Moutounet M., Cheynier V., Samson A., 2007. The influence of pH and late micro oxygenation on sourness, bitterness, and astringency of red wine. *8e Symposium d'oenologie de Bordeaux*, Bordeaux, France.

Nevoigt E., 2008. Progress in metabolic engineering of *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. 72, 379-412.

Pérez-Torrado R., Oliveira B.M., Zemančíková J., Sychrová H., Querol A., 2016. Alternative glycerol balance strategies among *Saccharomyces* species in response to winemaking stress. *Front. Microbiol.* 7, 435.

Remize F., Roustan J.L., Sablayrolles J.M., Barre P., Dequin S., 1999. Glycerol overproduction by engineered *Saccharomyces cerevisiae* wine yeast strains leads to substantial changes in by-product formation and to a stimulation of fermentation rate in stationary phase. *Appl. Environ. Microbiol.* 65, 143-149.

Sablayrolles J.M., Dubois C., Manginot C., Roustan J.L., Barre P., 1996. Effectiveness of combined ammoniacal nitrogen and oxygen additions for completion of sluggish and stuck wine fermentations. *J. Ferment. Bioeng*. 82, 377-381.

Salmon J.M., 2006. Interaction between yeast, oxygen and polyphenols during alcoholic fermentations: practical implications. LWT – *Food Sci. Technol.* 39, 959-965.

Salmon J.M., Ortiz-Julien A., 2007. Improvement of alcoholic fermentation in extreme conditions. *Bull. OIV* 914-916, 321-328.

Samson A., Escudier J.L., Bouissou D., Caillé S., 2009. Baisse du pH par procédé électro-membranaire. *In: Le Vin Rosé*, pp. 207-208. C. Flanzy, G. Masson, F. Millo (eds), Féret, Bordeaux, France.

Schmidtke L.M., Blackman J.W., Agboola S.O., 2012. Production technologies for reduced alcoholic wines. *J. Food Sci.* 77, R25-R41.

Sieczkowski N., Ortiz-Julien A., Garcia E., Samson A., Caillé S., Salmon J.-M., 2016a. Une nouvelle levure inactivée spécifique comme solution naturelle pour la maîtrise de l'oxygène et la conservation de la qualité des vins en élevage. Partie 1/2 : Mise au point et preuve de concept. *Rev. Œnol.* 159, 25-28.

Sieczkowski N., Ortiz-Julien A., Garcia E., Samson A., Caillé S., Salmon J.-M., 2016b. Une nouvelle levure inactivée spécifique comme solution naturelle pour la maîtrise de l'oxygène et la conservation de la qualité

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des vins en élevage. Partie 2/2 : Validation en situations pratiques de cave. *Rev. Œnol.* 160, 38-40.

Sire Y., Douçot S., Pull M.-J., Samson A., Moutounet M., Salmon J.-M., 2016. Activité polyphénol-oxydase du raisin : influence de la température et de différentes opérations. *Rev. Œnol.* 160, 25-28.

Soubeyrand V., Luparia V., Williams P., Doco T., Vernhet A., Ortiz-Julien A., Salmon J.M., 2005. Formation of micella containing solubilized sterols during rehydration of active dry yeast improves their fermenting capacity. *J. Agric. Food Chem.* 53, 8025-8032.

Tilloy V., Cadiere A., Ehsani M., Dequin S., 2015. Reducing alcohol levels in wines through rational and evolutionary engineering of *Saccharomyces cerevisiae*. *Int. J. Food Microbiol.* 213, 49-58.

Varela C., Kutyna D.R., Solomon M.R., Black C.A., Borneman A., Henschke P.A., Pretorius I.S., Chambers P.J., 2012. Evaluation of gene modification strategies for the development of low-alcohol-wine yeasts. *Appl. Environ. Microbiol*. 78, 6068-6077.

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