

LA RIOJA - APRIL 27-28, 2005

YEAST'S CONTRIBUTION TO THE SENSORY PROFILE OF WINE

MAINTAINING TYPICITY AND BIODIVERSITY
IN THE CONTEXT OF GLOBALIZATION

PROCEEDINGS
OF

LES XVII^{es} ENTRETIENS SCIENTIFIQUES LALLEMAND

LALLEMAND

FOREWORD

The role of yeast in winemaking is for much more than alcoholic fermentation. The impact of yeast on the sensory quality of wines became abundantly clear at the *XVII^{es} Entretiens Scientifiques Lallemant* held in La Rioja, Spain, on April 27 and 28, 2005. The technical meeting this year brought together researchers, winemakers and oenologists from eight countries to discuss advances in winemaking science and oenological practices related to the utilization of selected natural yeast in the production of wines. The nearly 200 attendees learned from the presentations and discussions on the sensory contribution of yeast, leading to a better understanding of its role in maintaining the typicity and biodiversity of wine in a context of globalization. Such issues as the sensory contribution of yeast, legislation regarding the use of yeast and its derivatives, research into genetically-modified microorganisms and their potential,

and the ecology of yeast dissemination were covered in the scientific papers presented.

Attendees were then invited to a round table discussion with 10 oenologists and winemakers from Europe and the New World who shared their perspectives on the practical aspects of utilizing selected natural yeast. They were concerned most by two issues: maintaining the natural character and typicity of wines, and offering consumers wines of faultless quality.

Not only do these technical meetings help attendees stay informed, the scientific presentations, the experiences of the winemakers and oenologists, and the comments from participants expressed at the *XVII^{es} Entretiens Scientifiques* will help Lallemant direct yeast research, production and communications to better respond to the increasingly specific and distinctive needs of the users of selected natural yeast.

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SELECTION AND POTENTIAL OF AUSTRALIAN *SACCHAROMYCES BAYANUS* YEAST FOR INCREASING THE DIVERSITY OF RED AND WHITE WINE SENSORY PROPERTIES

Jeffrey M. EGLINTON, I. Leigh FRANCIS and Paul A. HENSCHKE

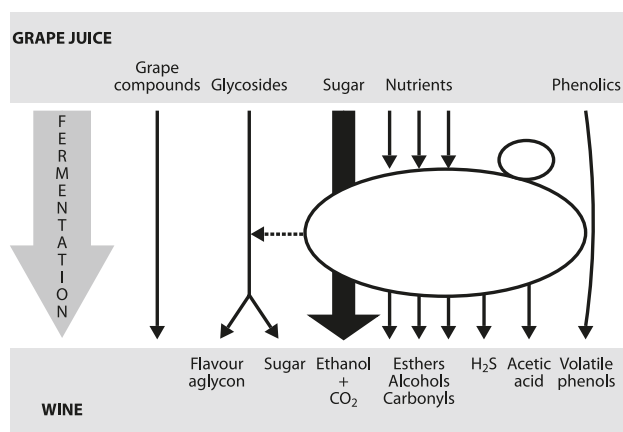
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The world market for wine, which presently exceeds 29 billion litres and represents hundreds of thousands of products contributed by over 34 countries, produces a global annual surplus exceeding five billion litres (Swiegers et al. 2005). This widening gap between wine production and consumption is intensifying competition and causing wine producers to innovate and create new ways to differentiate their wines in the ever more crowded global marketplace. Numerous tools and techniques (for example, grape cultivars and their management, must processing, fermentation yeast and conditions, secondary fermentations, wood treatment, and wine maturation and packaging) are available, or being developed, that can be applied across the process from vineyard to the bottle. Among them, the choice of fermentation yeast offers considerable potential, although this still remains a largely unexploited resource (Henschke 1997; Heard 1999; Lambrechts and Pretorius 2000; Howell et al. 2005).

The interaction between yeast and grape must is complex and involves a myriad of substrates and products, some of which have important sensory impact. Fig. 1 summarizes some of the more important interactions that are involved in the fermentative transformation of a relatively low flavoured substrate to a highly flavoured product. The nutrients present in grape must (carbon-, oxygen-, nitrogen-, sulphur- and phosphorus-containing compounds, vitamins, minerals and trace elements) not only provide all the factors necessary for growth but their metabolism, and especially that of the sugars and amino acids, generates a range of non-volatile (principally polyols and acids) and

volatile (esters, alcohols, aldehydes and ketones, fatty acids, sulphides and phenols) metabolites that contribute to the wine's taste and "fermentation bouquet" (Swiegers et al. 2005). In addition, yeast interacts with a variety of grape-derived flavour precursors, most notably glycosides, cysteinyl conjugates and phenolic compounds (Eglinton et al. 2004; Dillon et al. 2004; Ugliano et al. 2005 [these proceedings]; Howell et al. 2005; Swiegers et al. 2005). The genetic variability of the grape vine, the fermentation yeast(s) and fermentation conditions can potentially generate a very large array of flavour profiles.

FIGURE 1. The routes by which flavour diversity can be generated using yeast



Saccharomyces cerevisiae is the pre-eminent winemaking yeast, principally because it functions competitively in this physiologically challenging environment (Henschke 1997; Bauer and Pretorius 2000). In particular, this species is highly adaptable to a dramatically changing chemical and nutritional environment of initially high nutrient

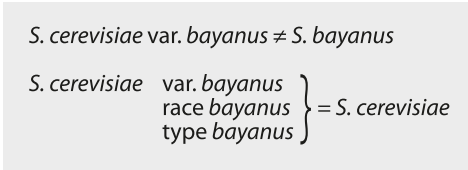
content to one of low nutrients and high concentration of metabolic end products, some of which, such as alcohol, can inhibit metabolic function. Well over 100 unique strains that have been selected by winemakers and scientists for producing desirable sensory properties, with many maintaining vineyard/regional or *terroir* characteristics, are commercially available. Whereas new technologies and biotechnologies are being developed around this yeast to extend its utility for winemaking (Pretorius 2000; Dequin 2001; Bisson 2004), a range of other yeast strains associated with grapes and wine are being evaluated for their potential to diversify and expand the scope of wine sensory properties. Of the 100 yeast genera that represent more than 700 species, only some 16 are associated with winemaking and represent huge genetic diversity (Pretorius et al. 1999; Fleet 2003).

Saccharomyces bayanus is one such yeast that shows considerable potential as an alternative (non-*S. cerevisiae*) winemaking yeast. *S. bayanus* is a member of the *Saccharomyces sensu stricto* group, which is dominated by alcohol-tolerant strains, such as *S. cerevisiae*. Although there is some debate about the composition of this taxon, it is now generally accepted that there are six species, namely *Saccharomyces bayanus*, *Saccharomyces cariocanus*, *Saccharomyces cerevisiae*, *Saccharomyces kudriavzevii*, *Saccharomyces mikatae* and *Saccharomyces paradoxus*. Strong genetic overlap of species within the group, despite the emergence of ever more sophisticated techniques for measuring genetic relatedness, ensure that the fate of two other species, *Saccharomyces pastorianus* and *Saccharomyces uvarum*, remains unclear (Rainieri et al. 2003; Nguyen and Gaillardin 2005). Indeed, the taxonomic status of the *S. bayanus* type strain, CBS 380, has recently been questioned (Nguyen et al. 2000 and 2005) and some researchers urge caution when considering the *S. bayanus* and *S. uvarum* groups (Rainieri et al. 2003). For the purposes of the work reported here, we will assume the existence of *S. bayanus* as a separate species and the isolates will be referred to as *S. bayanus*.

Although *S. bayanus* is as genetically distinct from *S. cerevisiae* as man is from mouse (depending on the genetic basis for the comparison) and, therefore, has some physiological and metabolic properties that differ significantly, its core properties are sufficiently similar that selected strains can successfully substitute for *S. cerevisiae* (Giudici et al. 1995; Feuillat et al. 1997; Zapparoli et al. 2003). There are few true *S. bayanus* strains commercially available to winemakers, although a natural hybrid of *S. bayanus* and *S. cerevisiae* (Lalvin S6U from Lallemant) is being used successfully.

The species, *S. bayanus*, should not be confused with a former species of the same name that was in common use until the early 1980s when reappraisal of the *Saccharomyces* genus resulted in the consolidation of 21 physiological species to the single species, *S. cerevisiae*, and the creation of four closely related genera (Kreger van Rij 1984). Although these 21 species had a variety of physiological and biochemical differences, they were inter-fertile and could produce viable progeny. In fact, the former *S. bayanus* species could only be differentiated from *S. cerevisiae* by the fermentation of galactose, a sugar which is not important in winemaking. The former *S. bayanus* species has, therefore, been reduced to a variety of *S. cerevisiae*, though some commercial producers still mistakenly refer to *S. cerevisiae* var. *bayanus* as *S. bayanus*. Because several strains of *S. cerevisiae* var. *bayanus*, most notably *Prise de Mousse* (IOC 18-2007) and EC1118, have well-known winemaking properties, many consider *S. cerevisiae* var. *bayanus* to represent a special group of wine yeast. Although there is no evidence to support this view, special care needs to be taken to differentiate the two types of yeast.

FIGURE 2. Dispelling some of the confusion that surrounds the nomenclature of *Saccharomyces bayanus* wine yeast



Isolation and identification of *Saccharomyces bayanus*

During a research project to isolate cryotolerant yeasts, several yeast strains were isolated from non-filtered, cold-stored grape juices that were vigorously fermenting at less than 4°C. The juices, which were divided into two portions, were allowed to complete fermentation at 1°C or 10°C. The fermentation properties, summarized in Table 1, show that these indigenous strains have strong fermentation capacity, not only at 10°C but also at 1°C.

TABLE 1. Fermentation characteristics of indigenous *S. bayanus* strains in cold-stored grape juice.

Property	Fermentation temperature	
	1°C	10°C
Time to completion (days)	200-250	30-40
Residual sugar (g/L)	15-33	0-5
Alcohol concentration (% v/v)	11.0-13.6	13.0-14.0

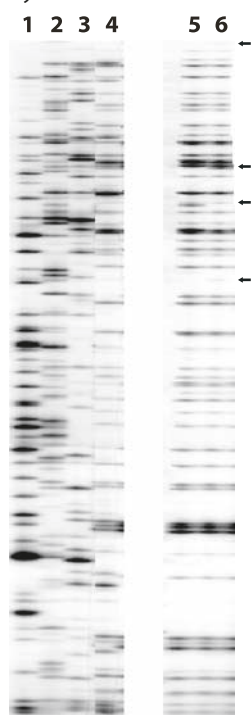
The strains isolated from the cold-stored grape juices were identified as *S. bayanus*, a species that is known to be cryotolerant. Yeast identification was confirmed by three

PCR-based techniques: restriction fragment polymorphism (RFLP) of the MET2 gene (Masneuf et al. 1996), intron splice site polymorphism analysis (de Barros Lopes et al., 1996) and amplified fragment length polymorphism (AFLP) analysis (de Barros Lopes et al. 1999) using the type strain, *S. bayanus* CBS 380, as the reference yeast.

The Australian Wine Research Institute has more than a dozen *S. bayanus* strains that have been isolated from grape juice or wine. AFLP characterization is used to characterize strains to ensure that we work with genetically diverse strains (Fig. 3).

FIGURE 3. An example of differentiation of yeast strains by AFLP analysis

The yeasts are (1) *S. paradoxus* CBS 432, (2) *S. cerevisiae* CBS 1171, (3) *S. pastorianus* CBS 1538, (4) *S. bayanus* CBS 380 and (5), (6) two *S. bayanus* isolates (see AWRI 1176 and AWRI 1375 below). Bands that differentiate between the two *S. bayanus* isolates are indicated with arrows.



While investigating cryotolerance in these strains, it was discovered that wines with interesting aroma and flavour attributes could be produced in fermentation trials. Soon, the cryotolerant behaviour of the strains had become of secondary importance to their potential as practical alternatives to *S. cerevisiae* strains for winemaking.

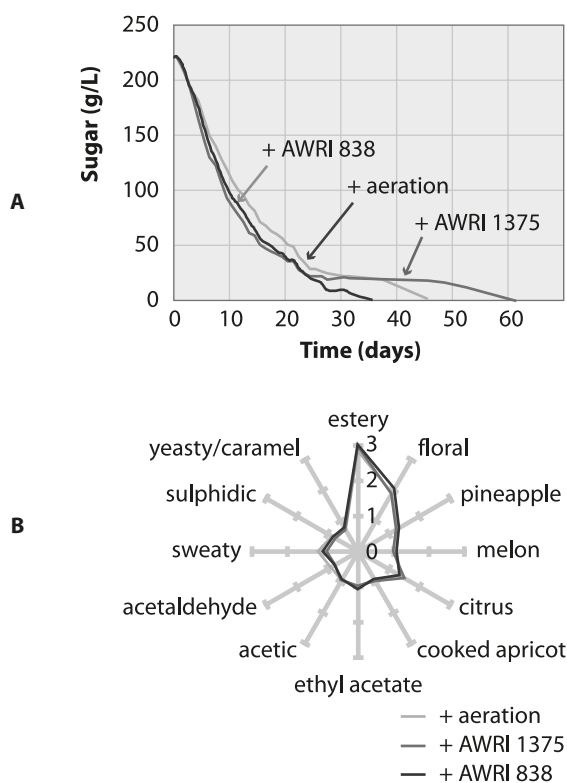
Winemaking characteristics of *Saccharomyces bayanus*

Of the *S. bayanus* isolates held in the Australian Wine Research Institute culture collection, the isolates AWRI 1176 and AWRI 1375, have been extensively characterized.

Unlike some other non-*S. cerevisiae* wine yeasts that have an impaired fermentative capacity, these two *S. bayanus* isolates are capable of fermenting typical grape juices to dryness. The strains often exhibit a lower overall fermentation rate than is typical for robust *S. cerevisiae* strains (such as Lalvin EC1118), which might be useful when low vigour ferments are preferred but could be undesirable if a short fermentation time is imperative. A slowing of fermentation rate by AWRI 1176 and AWRI 1375 toward the end of fermentation is sometimes observed but, in those cases, sequential inoculation with either *S. cerevisiae* or *S. bayanus*, or aeration achieved by pumping over, ensures a low residual sugar concentration without substantially affecting the aroma profile of the wine (Eglinton et al. 2005; Fig. 4).

FIGURE 4. Effect on the aroma profile of Chardonnay wine of different techniques to ensure complete fermentation

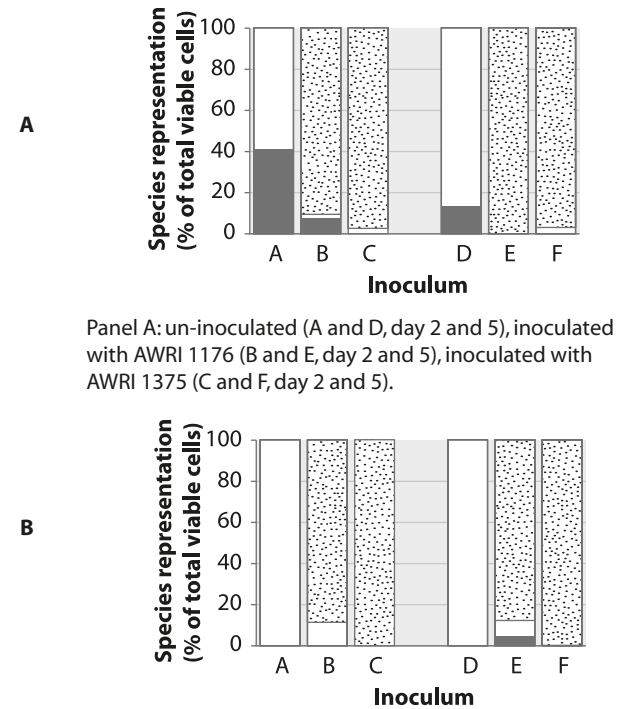
The techniques included sequential inoculation with *S. cerevisiae* AWRI 838 after two thirds of the sugar had been consumed, pumping over with some aerobic handling, and re-inoculation with a rescue culture of *S. bayanus* AWRI 1375. The wine was a difficult-to-ferment Chardonnay juice (pH 2.9, TA > 9 g/L, sterile processed and cellar bright). Panel A, fermentation kinetics. Panel B, 12 most important aroma descriptors for these wines as determined by sensory descriptive analysis.



Rapid domination (in terms of cell number) of the indigenous microflora by an inoculated strain is a characteristic of good wine yeast strains, and is essential to ensure that the proliferation of undesirable organisms is minimized

or that essential nutrients are not depleted by organisms other than those in the inoculum. In laboratory-scale ferments in a Shiraz must and commercial barrel-scale ferments in a Chardonnay juice, the inoculated AWRI 1176 and AWRI 1375 strains showed that they could dominate the yeast population from the early stages of fermentation such that they were the major (often the only) yeast present at the end (Fig. 5; Eglinton and Henschke 2002b). In control ferments, the indigenous or inoculated *S. cerevisiae* species rapidly dominated. The ability to dominate a ferment sets *S. bayanus* AWRI 1176 and AWRI 1375 apart from some other non-*S. cerevisiae* yeasts that are fermentation impaired and that cannot reliably conduct a fermentation.

FIGURE 5. Yeast species (% of viable population) present in Shiraz ferments after 2 and 5 days of fermentation (Panel A), and Chardonnay barrel ferments after 3 days and at the end of fermentation (Panel B).



Panel A: un-inoculated (A and D, day 2 and 5), inoculated with AWRI 1176 (B and E, day 2 and 5), inoculated with AWRI 1375 (C and F, day 2 and 5).

Panel B: inoculated with Levuline CHP (A and D, day 3 and eof*), inoculated with AWRI 1176 (B and E, day 3 and eof), inoculated with AWRI 1375 (C and F, day 3 and eof).

Non-*Saccharomyces* species (■), *S. cerevisiae* (□), *S. bayanus* (▨).

*eof = end of fermentation

The nutrient requirements of these *S. bayanus* strains have not yet been fully characterized. However, the response of these strains to oxygen and nitrogen during sluggish or stuck fermentation indicates that these strains have requirements similar to *S. cerevisiae*. Evolution of sulphidic aromas has been observed in some musts and their amelioration is generally achieved with diammonium phosphate addition. However, a high nitrogen demand was

recently demonstrated by chemostat characterization by the method of Julien et al. (2000) (A. Julien, personal communication). Thus, management of these strains could benefit from the practices that have proven useful for high nitrogen requiring *S. cerevisiae* strains (Blateyron and Sablayrolles 2000). That is, ensuring that the juice or must has at least 200-300 mg/L yeast assimilable nitrogen and that a short aeration step is included during fermentation.

S. bayanus strains can be used to produce wines with a different chemical composition than those made with conventional *S. cerevisiae* wine yeast strains (Table 2). *S. bayanus* AWRI 1176 and 1375 produce more glycerol and succinic acid than typical *S. cerevisiae* wine yeast strains, but less acetic acid and, sometimes, less ethanol (Table 3). The exact effect that glycerol has on the mouth-feel of wine remains unclear, but it is unlikely that the increased glycerol concentration has a direct effect on the palate structure or viscosity in *S. bayanus* wines because the concentration does not typically reach the published threshold at which it impacts on wine viscosity (20-25 g/L, Noble and Bursick 1984; Nurgel and Pickering 2005). Given the present interest from winemakers and consumers in low-alcohol wines, the capacity to form less alcohol during fermentation could be an important oenological trait of this species and warrants further investigation.

TABLE 2. General properties of *S. cerevisiae* and *S. bayanus* yeasts

Behaviour	Property	
	<i>S. cerevisiae</i>	<i>S. bayanus</i>
Fermentation temperature	10-35°C	6-30°C
Optimum growth temperature	> 30°C "mesophilic"	25-30°C often "cryotolerant"
Formation of: Acetic acid Ethanol Glycerol Malic acid Succinic acid	Low-high wide range wide range neutral/degrade low-medium	Low < <i>S. cerevisiae</i> > <i>S. cerevisiae</i> neutral/produce medium-high

S. bayanus AWRI 1176 and AWRI 1375 produce a profile of aroma- and flavour-active minor fermentation products that is different from that of typical *S. cerevisiae* wine yeasts (Table 4). These strains produce a high concentration of 2-phenylethanol, a trait that has been reported for other *S. bayanus* strains (Antonelli et al. 1999). Other alcohols that can be produced at a high concentration include iso-butanol and isoamyl alcohol (fusel-like/whiskey aromas). Of the volatile esters, 2-phenylethyl acetate (floral aroma) is typically produced in greater amount by *S. bayanus* yeast strains than by *S. cerevisiae* strains, as is isoamyl acetate (banana aroma) at low fermentation temperature, although we have not observed an elevated banana aroma

TABLE 3. Composition of Chardonnay wines made on small-scale using *S. cerevisiae* or *S. bayanus* yeasts (Eglinton et al. 2000)

Initial grape juice composition: sugar, 227 g/L; malic acid, 2.37 g/L; pH, 3.31; TA, 5.8 g/L.

Property	<i>S. cerevisiae</i> AWRI 838	<i>S. bayanus</i> AWRI 1176	<i>S. bayanus</i> AWRI 1375
Residual sugar (g/L)	0.6	0.1	0.1
Alcohol (% v/v)	13.1	13.1	13.3
pH	3.40	3.39	3.38
Titrateable acidity (g/L)	6.8	6.5	6.5
Acetic acid (g/L)	0.43	0.10	<0.05
Glycerol (g/L)	5.1	8.6	7.9
Malic acid (g/L)	2.25	1.85	2.04
Succinic acid (g/L)	0.50	1.00	1.07

in Chardonnay wines made at low temperature with our isolates. Ethyl lactate and diethyl succinate also are generally more abundant in wines made with *S. bayanus*.

TABLE 4. Some volatile compounds in Chardonnay wine made using *S. cerevisiae* or *S. bayanus*.

Odour activity values (OAV) represent the ratio of analyte concentration to aroma threshold measured in the same matrix. The OAV for compounds marked with an asterisk are significantly different between the two yeasts.

Aroma compound	Aroma	Threshold ($\mu\text{g/L}$)	AWRI 838 OAV	AWRI 1375 OAV
ethyl octanoate*	sweet, fruity	2	993	815
ethyl hexanoate*	fruity, apple	5	251	144
ethyl 2-methyl butanoate*	sweet, fruity	1	0.1	26
ethyl propanoate	fruity	14	21	24
2-phenylethanol*	rose	7500	2	11
ethyl 2-methyl propanoate*	fruity	15	2	10
ethyl 3-methyl butanoate*	fruity, berry	3	4	9
ethyl butanoate*	fruity, sweet	20	17	8
3-methyl butyl acetate*	fruity, banana	30	18	8
ethyl decanoate	fruity, soap	200	3	3
Acetic acid	acetic, vinegar	175000	2	2

Chemical analysis of aroma- and flavour-active compounds cannot yet give a complete picture of the sensory properties of a wine. Quantitative sensory descriptive analysis is a powerful tool for discriminating between wines made us-

ing different yeasts. Chardonnay wines made with *S. bayanus* are generally characterized by more complex aromas and a less dominant fruity fermentation bouquet (Table 5 and Fig. 6; Eglinton et al. 2000). Some of the different aromas that are characteristic of AWRI 1176 and AWRI 1375 are cooked orange peel, apricot, honey, yeasty and nutty, which are considered by some experienced judges as positive contributors to wine aroma. We acknowledge that these complexing aromas will not be considered positive by all consumers or be suited to all wine styles. In Cabernet Sauvignon wines that were made under pilot winery-scale (750 kg) conditions in 2001, *S. cerevisiae* wines were described as cherry, plum and green, while *S. bayanus* wines that were made with the same fruit were characterized by the attributes raspberry, cherry, apricot, liquorice, herbal and earthy. The palate of *S. bayanus* wines often consists of more “developed” flavours than control wines, but the differences have not been rigorously assessed by quantitative descriptive analysis.

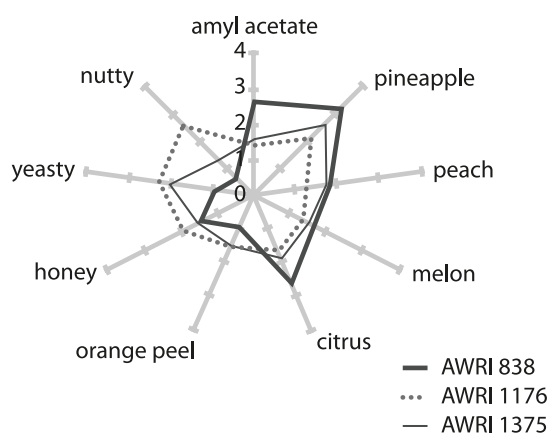
TABLE 5. Aroma descriptors for commercial Chardonnay wine made using *S. cerevisiae* or *S. bayanus*.

Descriptors that were considered more important are shown in *italics*.

Yeast	Aroma description
<i>S. cerevisiae</i> AWRI 838	<i>estery, pineapple, peach, floral, ethyl acetate</i>
<i>S. bayanus</i> AWRI 1176	<i>estery, pineapple, peach, floral, melon, apricot, honey, nutty</i>
<i>S. bayanus</i> AWRI 1375	<i>estery, pineapple, citrus/lime, floral, apricot, honey, nutty</i>

FIGURE 6. Sensory descriptive analysis of the aroma of Chardonnay wine

The values are mean ratings for nine important aroma attributes in Chardonnay wine made with *S. cerevisiae* AWRI 838, *S. bayanus* AWRI 1176 or *S. bayanus* AWRI 1375. The values are the mean score given by 13 judges to triplicate wines on two separate occasions.



The mouthfeel of wines made with *S. bayanus* AWRI 1176 or AWRI 1375 consistently appears to be fuller than that of wines made with control strains of *S. cerevisiae*, often showing greater weight and texture with lower apparent acidity and greater fruit persistence. *S. bayanus* wines display a distinct viscosity, but we do not believe that the increased “fullness” is due directly to the higher concentration of glycerol in these wines. Polysaccharides and other high molecular weight compounds could contribute significantly to the palate characteristics, and analysis of some of these compounds forms part of our ongoing investigation of these yeast strains. The texture of the palate was different in young Cabernet Sauvignon wines (2002) made using *S. bayanus*, being rated lower by a sensory descriptive analysis panel in the attributes velvet, drying and pucker, but higher in the attributes grainy and silky, when compared to wines made from the same fruit using *S. cerevisiae*.

Young Cabernet Sauvignon wines made with AWRI 1375 or *S. cerevisiae* AWRI 838 generally exhibit an obvious difference in colour on visual inspection (Fig. 7; Eglinton and Henschke 2003). The wines made in 2001 and 2002 with AWRI 838 were purple, while those made with AWRI 1375 were more red, with less purple hue, which gave the *S. bayanus* wines a more “aged” appearance. The colour difference was confirmed by spectrophotometry, with the *S. bayanus* wines having greater colour density (A420 + A520) and greater colour hue (A420/A520) than *S. cerevisiae* wines (Table 6). Analysis of wine phenolics by high performance liquid chromatography revealed some differences resulting from fermentation with the different yeasts, and we are currently investigating the chemical basis for the observed difference in wine colour. Although it is unlikely, we cannot discount that the greater colour density in wines made with *S. bayanus* was due, in part, to the slightly longer time on skins before pressing (one day longer than *S. cerevisiae* wines in 2002) as a result of the slower fermentation rate of *S. bayanus* AWRI 1375. We will also observe how the colour differences respond to aging of the wines in the future.

FIGURE 7. Colour of 2001 Cabernet Sauvignon wine made with *S. cerevisiae* or *S. bayanus*.

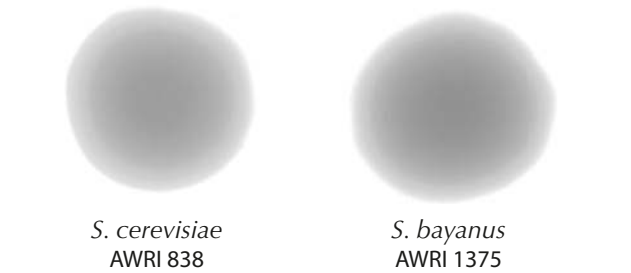


TABLE 6. Basic colour properties (colour density and colour hue) of Cabernet Sauvignon wines made with *S. cerevisiae* AWRI 838 or *S. bayanus* AWRI 1375 during the 2001 and 2002 vintages.

Values are the mean ± SD of triplicate fermentations.

Attribute	2001		2002	
	<i>S. cerevisiae</i>	<i>S. bayanus</i>	<i>S. cerevisiae</i>	<i>S. bayanus</i>
Colour density (A420 + A520)	6.8 ± 0.5	7.5 ± 0.3	8.5 ± 0.3	9.8 ± 0.2
Colour hue (A420 / A520)	0.64 ± 0.01	0.73 ± 0.01	0.74 ± 0.02	0.77 ± 0.01

Harnessing the potential of *Saccharomyces bayanus*

Despite the positive attributes of the strains AWRI 1176 and AWRI 1375 for some applications, we recognize that these strains can be regarded as “first generation” and might not represent the best possible *S. bayanus* strains for winemaking. We should remind ourselves that the search for *S. cerevisiae* strains commenced more than three decades ago and a tremendous human resource can be required to find robust production strains. Nevertheless, these strains demonstrate the great resource that new genetic material can offer. Further, not only has this project developed several novel strains, it has enhanced our ability to understand the biochemical mechanisms involved in the transformation of grape nutrient and flavour precursors.

The maximum winemaking potential of *S. bayanus* could be found in its use as a partner in mixed culture fermentation, either as a co-fermenter with *S. cerevisiae* or as an initial inoculum that is followed late in fermentation by an *S. cerevisiae* wine yeast. At least one commercial winery has achieved good results by finishing *S. bayanus* fermentations with *S. cerevisiae* at low (< 2°Be) sugar concentration.

No matter how they are used, *S. bayanus* strains offer winemakers an additional tool for differentiating their products. Harnessing their potential is about using carefully selected strains in a controlled manner to achieve the aroma and flavour diversity that winemakers are striving for, which is now a commercial reality for at least one of our strains (Clancy 2004).

Acknowledgments

Investigating the potential of AWRI 1375 and AWRI 1176 for winemaking has provided an ideal opportunity for a collaborative research effort among the Institute's microbiologists, molecular biologists and analytical and sensory chemists, together with the Australian wine industry. We are grateful to the Hardy Wine Company, Orlando Wyndham and Yalumba for the generous supply of premium grapes or for conducting pilot-scale fermentation trials. We are especially grateful to those winemakers who have performed winery evaluations of these yeasts and made their results available. Markus Herderich, leader of the AWRI Tannin Project, has generously donated his expertise and fruitful discussions regarding analysis of wine phenolics and colour. We thank the staff and students of The Australian Wine Research Institute who participated on the sensory panels and in winemaking, and the Molecular Biology team led by Miguel de Barros Lopes (now at the University of South Australia) for help with genetic characterization of yeast strains. This work was financially supported by Australia's grapegrowers and winemakers through their investment body, the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government.

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CREATION OF DIFFERENT VINIFICATION CONCEPTS USING THE GEISENHEIM YEAST FINDER

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Summary

The use of selected yeast starter cultures is highly recommended to avoid the quality-lowering effects of wild yeasts and bacteria. Selected yeast produced industrially should not only prevent the growth of unwanted microorganisms, they should help transfer the potential quality of the grape must into the actual quality of a future wine.

Fermentation conditions vary from winery to winery due to differences in the viticultural management, pressing systems, must clarification and fermentation temperature, and so on. Because of these differences, numerous yeast strains are available on the market. However, winemakers are often confused, and do not know how to select the right strain as the technical information sheet for a given yeast strain gives no or little information about the requirements of the strains in respect to nutrient content, fermentation temperature, length of fermentation, an eventual malolactic fermentation, etc., in order to display the advertised positive properties.

We have developed a computer program that helps overcome this situation by asking the winemaker simple but important questions about how the must has been produced and the desired temperature for fermentation. With this information, the software first calculates the potential nutrient content of the must and then proposes most suitable yeast strains. These recommendations by computer are possible only because we have successfully convinced major yeast producers to publish the nutritional and technical demands of their yeast strains according to a standardized chart that we have also developed.

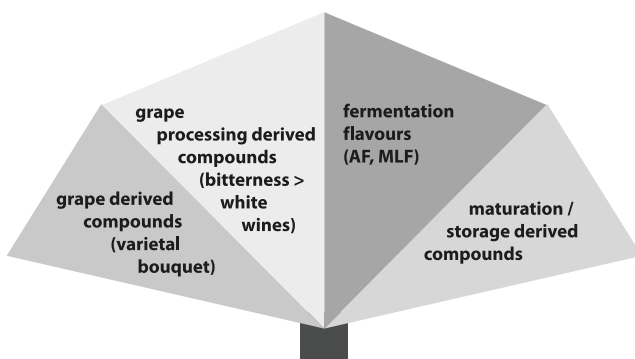
With this first step towards worldwide harmonization of yeast strain descriptions, winemakers now have a powerful electronic tool to choose the right yeast strains for their individually prepared grape musts (www.geisenheimer-hefefinder.de).

Origins of wine bouquet

The wine bouquet is composed of several hundred aromatic compounds. These huge numbers of substances are often grouped according to their origin (Fig. 1):

- Grape-derived compounds delivering the varietal character
- Grape-processing-derived compounds, such as specific phenolic compounds stemming from the harsh treatment of grapes, resulting in a bitter taste in white wines
- Fermentation flavours produced either by yeasts during the alcoholic fermentation (AF) or by lactic acid bacteria during malolactic fermentation (MLF).

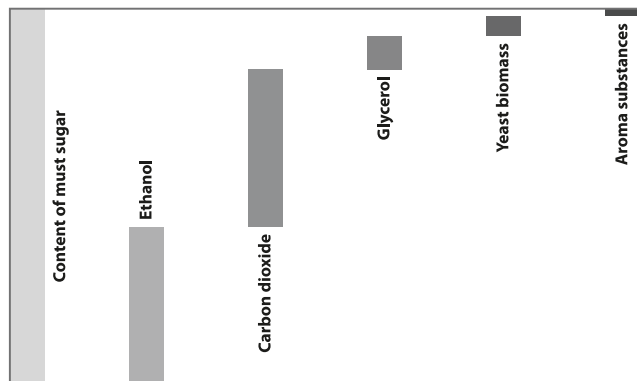
FIGURE 1. Origins of wine bouquet



The impact of each aroma group can be directly influenced by the winemaker by choosing the right time to pick the grapes (physiological ripeness), gentle grape processing techniques, targeted use of selected yeast and fermentation conditions, and by implementing suitable wine storage conditions. The latter, especially, has a tremendous influence on the fermentation flavour as the yeast flavours are not stable over time.

These compounds represent only a minor fraction of the products derived mainly from the conversion of must sugar (Fig. 2), but they build up, becoming a major factor when wine consumers decide to buy or not to buy a given wine. A pleasing wine flavour is the best entrance for wine consumption, and yeast-derived fermentation flavours are often the “door openers.” Therefore, it is of huge importance to choose the right yeast strain for a given grape must.

FIGURE 2. Actual balance of must sugar fermentation



Targeted use of selected yeast strains

The use of yeast starter cultures is highly recommended to avoid the quality-lowering effects of wild yeasts and bacteria. Selected yeast produced industrially should not only prevent the growth of unwanted microorganisms, they should help transfer the potential quality of the grape must into the actual quality of a future wine.

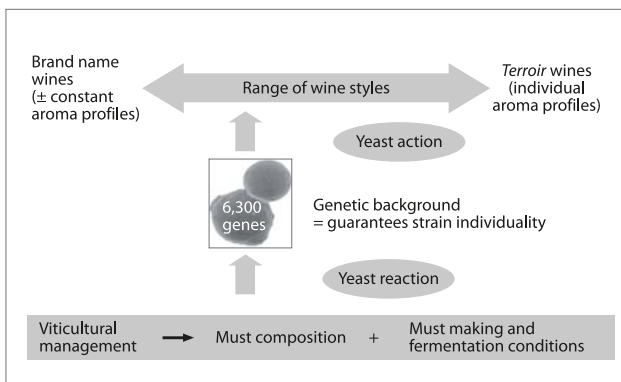
However, fermentation conditions differ from winery to winery due to differences in viticultural management, pressing system, must clarification and fermentation temperature.

Strains of the yeast species *Saccharomyces cerevisiae* may show a wide spectrum of properties in respect to the qualitative and quantitative formation of flavour compounds. More than 6,300 genes constitute a genetic reservoir that guarantees strain individuality which can be exploited. Fig. 3 shows the yeast cell as having the central role in flavour production during fermentation. However, yeast cells always react to the predominating environmental conditions, which are the must composition and the fer-

mentation conditions. Therefore, it is necessary to know as much as possible about the properties of yeast strains, but it is at least equally important to know exactly under what conditions these yeast properties are really displayed.

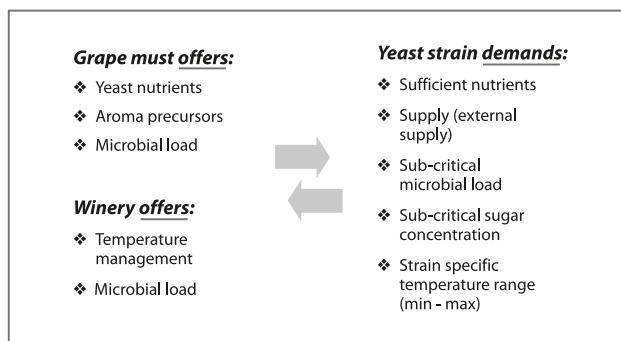
This knowledge then can usefully be applied for the creation of a broad range of wine styles, from brand name wines with more or less constant aroma profiles for every vintage, to *terroir* wines with more individual aroma characters (Fig. 3).

FIGURE 3. Targeted use of selected yeast strains



The efficient use of yeast strain properties is the final result of what the yeast strains are demanding and the grape musts are offering. This is illustrated in Fig. 4: grape musts offer the yeast nutrients, aroma precursors and sometimes an unwanted microbial load; the winery offers must-preparing techniques and temperature management. On the other hand, the yeasts demand sufficient nutrient supply (sometimes only through external additions), sub-critical microbial load, sub-critical sugar concentration and a strain-specific temperature range.

FIGURE 4. Efficient use of yeast strain properties



The better the “offers” and “demands” fit together, the fewer fermentation management problems occur.

Due to this complex situation and also due to the fact that more than 120 yeast strains are available on the German market, winemakers get confused about the important question: How to find the right strain as the technical information sheet for a given yeast strain gives no or only

incomplete information about the demands of the strain in respect to nutrient content, fermentation temperature, length of fermentation, eventual malolactic fermentation, etc., in order to develop the advertised positive properties.

Steps towards the Geisenheim Yeast Finder

The aim was to develop a computer program that helps winemakers overcome this situation. It was framed in a system where the winemaker is asked simple but important questions about how the grapes and the grape musts have been produced and under what conditions the fermentation will be performed.

With this information, first the software calculates the potential nutrient content of the must and then proposes the most suitable yeast strains with regard to fermentation conditions.

The following three steps were undertaken to establish the system:

Step 1: Development of a yeast data sheet

Development of a data sheet that monitors physiological yeast properties and the nutritional/environmental demands of the yeast strain.

Step 2: Development of a grape/must data sheet

Development of a data sheet that monitors grape production (viticultural factors), grape must production (oenological factors) and yeast user expectations about intensity of flavour formation during alcoholic fermentation.

Step 3: Development of the software package

Development of intelligent rules that combine yeast user demands for distinct yeast properties, as well as yeast strain demands for proper nutritional supply, and technical fermentation factors such as temperature control.

Yeast data sheet

The properties of commercial yeast strains are described as far as the individual yeast producer wants to have the yeast strains described. Normally distinct yeast properties are displayed only under certain nutritional conditions, i.e., sufficient quantity of nitrogen, or certain fermentation conditions, i.e., temperature, which have to be precisely investigated by the yeast producer and properly communicated to the yeast users.

After intensive discussions with yeast producers over the past two years and an increasing mutual recognition of the needs and demands of both parties (the yeast producer and the yeast user), it was possible to establish a basic

data sheet that is now used by yeast producers to describe their strains.

Now, for the first time ever, commercial yeast cultures are described on the same formal sheet. These harmonized yeast strain descriptions are available at www.forschungsanstalt-geisenheim.de, on the Web page of the “Fachgebiet Mikrobiologie und Biochemie” and shown under “Datenblätter Handelshefen”. (Unfortunately, available only in German at this time.)

Although this harmonization was a big step forward in terms of consumer information, other steps must follow to sharpen the description of specific yeast demands – for example, the concentration of nitrogen compounds that must be available and according to which method the content of these compounds must be analyzed.

As a result of the high number of yeast strains at this Web site, many yeast users felt uncertain how they should decide which yeast strain is the best for their specific wine-making conditions. These uncertainties created a need for a guiding system that eases the labour intensive work of comparing more than 100 yeast data sheets.

It rapidly became clear that only an electronic tool could shorten this decision process.

Grape/must data sheet

Knowing the specific conditions that yeast strains need as prerequisites for pleasant metabolic activity automatically creates the necessity for increased knowledge about the nutritional situation in a given grape must to fulfil these yeast demands. This information must be provided from two sides: first, by knowing the viticultural situation under which grapes were formed, and second, by knowing which oenological procedures were applied to press the grapes and clarify the must. In addition, and before any choice among yeast strains can be made, it must also be known at which temperature fermentation will be performed and what flavour intensity the fermentation bouquet should display.

While yeast producers had to supply data about their yeast strains, it is now the yeast user's turn to deliver information about the vinicultural aspects. To get this information, a data sheet was developed where potential yeast users fill in such details as grape variety, stress factors (lack of water, heat, green cover, etc.), yield, must weight, pressing system, must clarification system and intensity of must clarification, desired fermentation temperature and flavour intensity. Although there are a few questions to answer, it is still quite easy and fast, as possible answers

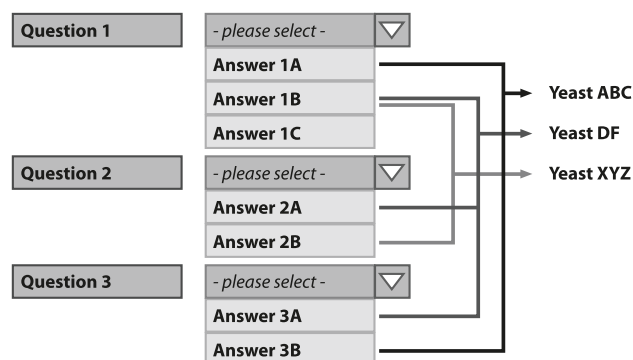
for each question are already displayed and the user must simply select the right one.

Software package

Once we had completed the yeast data sheets and the blank grape/must data sheet, the next important step was the development of an electronic tool that links the answers from the grape/data sheet with those on the yeast data sheet, and then select the yeast strains from the database that best fulfil the conditions in the given grape must and the intended fermentation procedure.

Fig. 5 shows the basic rules and connections that link the different elements between questions that the yeast user must answer (concerning the making of the grape must) and the answers that were given by the yeast producers to characterize their strains. With this rule-based principle, all answers from the yeast user concerning the grape must are compared with all yeast data sheets, and the degree of accordance established.

FIGURE 5. Rule-based reasoning process



Practical use of the expert system

The electronic tool is simple to use in a step-by-step procedure (as mentioned above, the system is currently available in German only at www.geisenheimer-hefefinder.de).

Winemakers interested in optimal must fermentation answer 17 questions, simply by accepting one of the possible answers. That's all. Then the system automatically searches for the most suitable yeast strains and shows their trade names on the screen. In this final step, the software displays the yeast strains either in decreasing order of strains within the same producer company or in an absolute ranking over all yeast manufacturers.

Once the recommended strains are known, the user can additionally open the relevant yeast data files to find out more about the yeast properties and about the yeast strain demands in terms of nutrient supply.

However, the final decision and responsibility for which strain(s) should be used lies exclusively with the yeast user.

Fig. 6a and Fig. 6b demonstrate the influence of the preferred fermentation temperature and the ranking of the yeast strains thereby influenced. The result in Fig. 6a was based on temperatures below 16°C, while in Fig. 6b fermentation temperature was said to be above 18°C.

Conclusions and future tasks

To our knowledge, the "Geisenheim Yeast Finder" represents the first Internet-accessible electronic guide to selecting specific individual yeast strains from among the commercially available yeast strains. This finder is based on a newly developed data sheet used worldwide by yeast producers to describe their yeast strains in the same manner for the first time.

However, some improvements are still necessary, including:

- Sharpening the yeast description profiles;
- Sharpening the yeast user's questionnaire;
- Establishing a scenario to check yeast strain behaviour independently and within reproducible test systems (definite media, micro-vinifications, etc.).

This system also needs to be adapted to other winemaking climate conditions and translated into more languages.

FIGURE 6A. Example 1 – Impact of fermentation temperature on yeast ranking

Geisenheimer Hefefinder

>Weinkategorie wählen >Daten eingeben >Gefundene Hefen

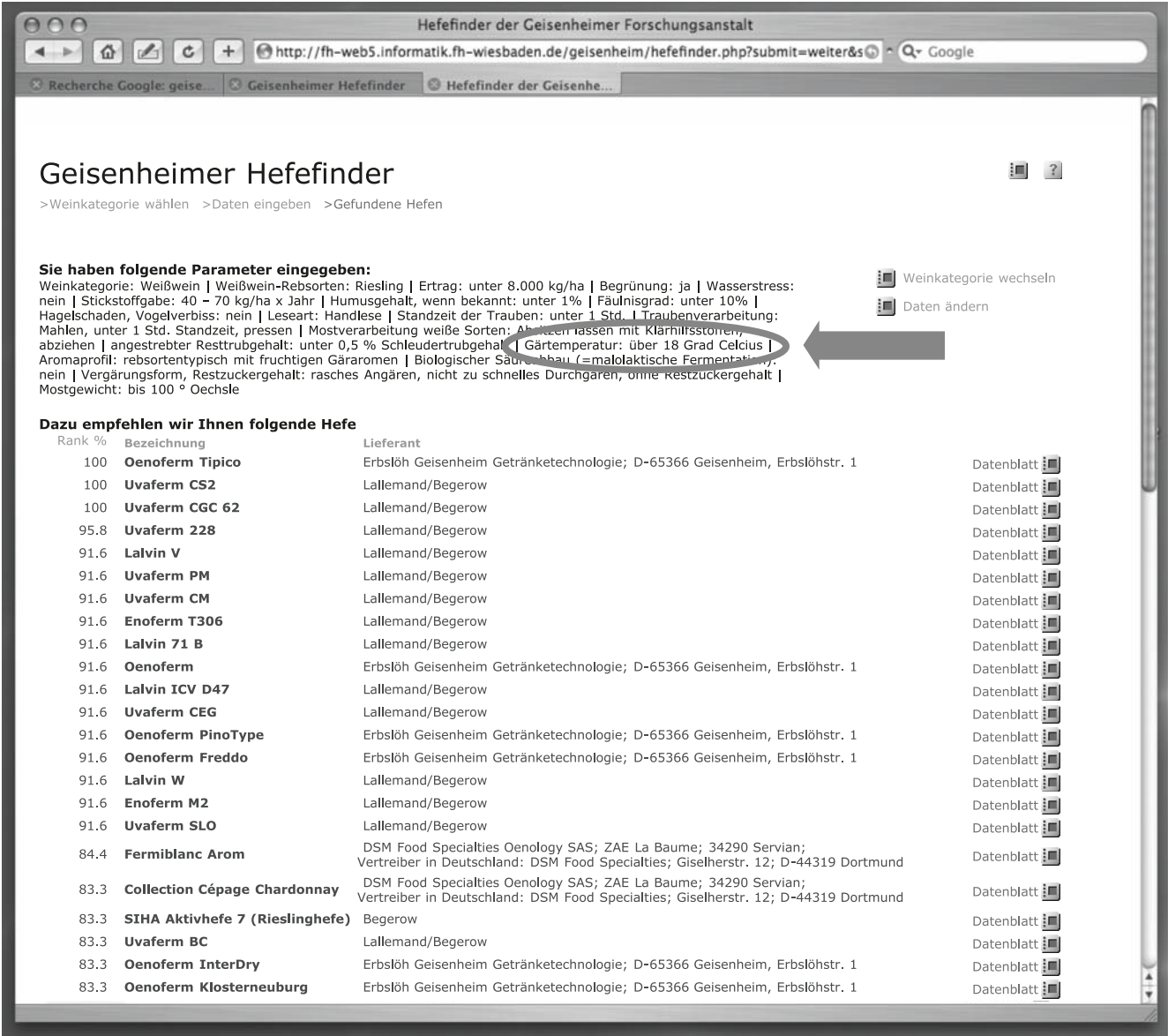
Sie haben folgende Parameter eingegeben:

Weinkategorie: Weißwein | Weißwein-Rebsorten: Riesling | Ertrag: unter 8.000 kg/ha | Begrünung: ja | Wasserstress: nein | Stickstoffgabe: 40 – 70 kg/ha x Jahr | Humusgehalt, wenn bekannt: unter 1% | Fäulnisgrad: unter 10% | Hagelschaden, Vogelverbiß: nein | Leseart: Handlese | Standzeit der Trauben: unter 1 Std. | Traubenverarbeitung: Mahlen, unter 1 Std. Standzeit, pressen | Mostverarbeitung weiße Sorten: Abkühlen lassen mit Klärhilfsstoffen, abziehen | angestrebter Restzuckergehalt: unter 0,5 % Schleudertrubgehalt | **Gärtemperatur: unter 16 Grad Celcius** | Aromaprofil: rebsortentypisch mit fruchtigen Gäraromen | Biologischer Säureabbau (=malolaktische Fermentation): nein | Vergärungsform, Restzuckergehalt: rasches Angären, nicht zu schnelles Durchgären, ohne Restzuckergehalt | Mostgewicht: bis 100 ° Oechsle

Dazu empfehlen wir Ihnen folgende Hefe

Rank %	Bezeichnung	Lieferant	Datenblatt
100	Uvaferm CGC 62	Lallemand/Begerow	Datenblatt
100	Lalvin W	Lallemand/Begerow	Datenblatt
100	Uvaferm CS2	Lallemand/Begerow	Datenblatt
95.8	Uvaferm 228	Lallemand/Begerow	Datenblatt
91.6	Lalvin R2	Lallemand/Begerow	Datenblatt
91.6	Enoferm T306	Lallemand/Begerow	Datenblatt
91.6	SIHA Cryarome	Begerow	Datenblatt
91.6	Fermicru VB1	DSM Food Specialties	Datenblatt
91.6	Uvaferm SLO	Lallemand/Begerow	Datenblatt
91.6	Uvaferm CEG	Lallemand/Begerow	Datenblatt
91.6	Lalvin V	Lallemand/Begerow	Datenblatt
91.6	Uvaferm PM	Lallemand/Begerow	Datenblatt
91.6	VIN 13	Anchor Yeast, Südafrika / C. Schliessmann GmbH & Co. KG; Auwiesenstr. 5; 74523 Schwäbisch Hall	Datenblatt
91.6	Oenoferm Tipico	Erbslöh Geisenheim Getränketechnologie; D-65366 Geisenheim, Erbslöhstr. 1	Datenblatt
91.6	Uvaferm CM	Lallemand/Begerow	Datenblatt
91.6	Oenoferm Freddo	Erbslöh Geisenheim Getränketechnologie; D-65366 Geisenheim, Erbslöhstr. 1	Datenblatt
91.6	Oenoferm Bouquet	Erbslöh Geisenheim Getränketechnologie; D-65366 Geisenheim, Erbslöhstr. 1	Datenblatt
83.3	Lalvin W46	Lallemand/Begerow	Datenblatt
83.3	Lalvin ICV D47	Lallemand/Begerow	Datenblatt
83.3	NT 45	Anchor Yeast, Südafrika / C. Schliessmann GmbH & Co. KG; Auwiesenstr. 5; 74523 Schwäbisch Hall	Datenblatt
83.3	Lalvin EC 1118	Lallemand/Begerow	Datenblatt
83.3	Anaferm 5	Lallemand /Verband Deutscher Weinanalytiker	Datenblatt
83.3	Enoferm M2	Lallemand/Begerow	Datenblatt

FIGURE 6B. Example 2 – Impact of fermentation temperature on yeast ranking



IMPACT OF YEAST ON THE VARIETAL AROMA OF WINE

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Introduction

For quite a long time, the understanding of the chemical base of wine aroma progressed too slowly due to its amazing complexity and to the weaknesses of the scientific approaches used. For years, the list of aroma chemicals identified in wine grew, to reach a figure near 1000. However, such overwhelming chemical information did not bring about a clear understanding of what chemicals were really behind the different wine aroma nuances, and most people thought that such complexity meant that the aroma of wine was just beyond scientific understanding.

In the last few years, however, a series of scientific findings together with the use of rigorous sensory-based analytical strategies have made it possible to screen the truly aroma-active compounds of wine (not so many, after all), to re-constitute the aroma of some not very complex wines^{1, 2, 3} or wine aroma fractions⁴, and to develop models able to accurately predict most wine aroma nuances from the knowledge of the wine content in a number of key odourants^{5, 6}. Such knowledge is in turn steering new research directed to understand wine aroma formation and optimization^{7, 8, 9}, and is expanding our understanding of the role of yeast in the development of the most appreciated varietal aroma nuances of wine.

This presentation will provide an overview of such recent findings, giving an updated classification of wine aroma compounds according to their capacity to impact the aroma of some wines. The concept of varietal aroma will be further expanded to show the different ways by which the genetic specificities of a grape variety can influence the

aroma of wine. Lastly, the role played by yeast in varietal aroma formation will be specifically discussed to show new ways of understanding the biodiversity of yeast and to develop rational criteria for selecting yeasts able to produce better, richer and more diverse wines expressing the full potential of a given grape.

A classification of wine aroma compounds

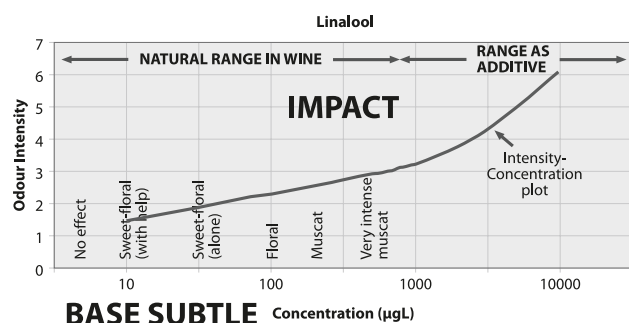
From our point of view, the most useful criterion to classify wine aroma compounds is according to their potential ability to really impact the aroma of a given wine, that is to say, according to their potential ability to cause any recognizable sensory difference between wines. According to this criterion, wine aroma compounds can be classified into the following categories.

1. Impact compounds. The compounds that can effectively transmit their specific (positive) aroma nuance to a given wine. An example: linalool.
2. Impact groups of compounds. These are families of compounds usually having similar chemical structures (chemical homologous series) with quite close odour properties, and that can impart to the aroma of a wine the specific notes of the family. An example: γ -lactones.
3. Subtle compounds or families. These are the compounds or groups of compounds that fail to transmit their specific aroma nuances to the wine, but contribute decisively to the development in wine of some secondary-generic aroma nuance (for instance fruity, sweet, etc.).

- Compounds forming the base of wine aroma. These are the compounds, present in all wines at concentrations above their corresponding odour thresholds which, however, are no longer perceived as single entities because their aroma is fully integrated to form the complex concept of wine aroma.
- Off-flavours. These are the compounds whose presence brings about a decrease in the general aroma quality of wine.

It is extremely important to note that this classification is dynamic and based on potentiality rather than on a fixed aroma property. The following example will illustrate this point.

FIGURE 1. Odour intensity versus concentration plot for linalool



The plot in Fig. 1 shows the odour intensity vs. concentration relationship for linalool. Superimposed in the plot are the sensory descriptors to which this compound can contribute in wine depending on its concentration. As can be seen, below 10 ppb this compound is not odour-active in wine. Between 10 ppb and 20 ppb it can be perceived, but only if it is reinforced by the presence of some other compounds with similar aroma. In this case, its contribution to the aroma of wine is generic and limited to an unspecific sweet-floral aroma nuance and will be classified as base. Between 20 ppb and around 50 ppb, it reaches enough power that it can be perceived independently of the presence of other compounds. However, it only communicates to wine a generic sweet-floral note. Between 50 ppb and 120 ppb, it is responsible for a clear floral odour nuance. In both cases it would act as a subtle compound. Only beyond this point does the note become muscat and the compound acts as a genuine impact compound.

Keeping in mind these precisions, the compounds that, to our knowledge, can act as genuine impact compounds in wine are the following:

- Linalool
- Cis-rose oxide
- 4-methyl-4-mercaptopentan-2-one
- 3-mercaptohexanol

- 3-mercaptohexyl acetate
- Isoamyl acetate
- (E)-whisky lactone
- Sotolon
- Diacetyl

To these nine compounds, it is necessary to add another seven families of compounds that can also act as impact groups of compounds. These families include:

- γ -lactones (γ -octa, nona, deca, undeca and dodecalactones)
- Volatile phenols (guaiacol, eugenol, isoeugenol, 2,6-dimethoxyphenol and 4-allyl-2,6-dimethoxyphenol)
- Vanillin and related compounds (vanilline, ethyl vanillate, methyl vanillate, acetovanillone and propiovanillone)
- Burnt sugar compounds (furaneol, homofuraneol, maltol, sotolon)
- Fusel alcohol acetates (isobutyl, isoamyl, phenylethyl, hexyl acetates)
- Fatty acid ethyl esters
- Fino aldehydes (isobutyraldehyde, 2- and 3- methylbutyraldehydes)

The base of the aroma of wine is formed by the following compounds or groups of compounds:

- Fatty acids
- Fatty acid ethyl esters
- Fusel alcohols
- Fusel alcohol acetates
- Isoacids
- Isoacid ethyl esters
- β -damascenone
- Acetaldehyde
- Methionol

It can be observed that there is some overlapping between categories. This happens because in some particular wines, one of the families of compounds in the base becomes especially important. For instance, in many young white wines fatty acid ethyl esters and fusel alcohol acetates become impact families of compounds. Leaving aside these cases, compounds in the base cannot cause important differences between wine types or wine qualities, although, of course, small variations can always be observed. The most important differences between wine types and wine qualities are due to compounds or families of compounds classified as potential impact compounds.

There are some other compounds that can also be impact compounds but whose role in wine is not clearly positive. These are the ethyl phenols, methoxypyrazines, DMS or methional. To our knowledge, these compounds cause some positive odour nuances of wine (such as woody and fruity characteristics) to decrease, and have odours that most often are not tolerated in wine. From this point of view, they are off-flavours. However, there are some types of wine in which the particular odour nuances of these compounds are well appreciated, at least by the local consumers.

The role of grape variety in wine aroma

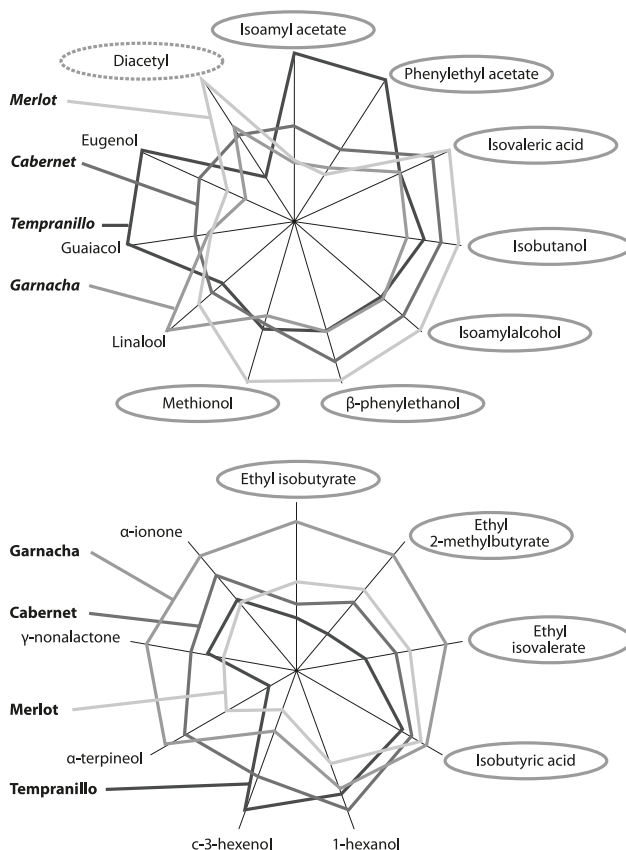
The genetic specificity of a given grape variety has different ways to make the aroma of a wine differentiable from the wines made with other grape varieties. The most obvious way is by directly producing huge amounts of some odorants that in most other grape varieties are absent or present at reduced levels. This is the case of terpenols. All grape varieties have the ability to produce small amounts of these compounds, but only muscat and muscat-related varieties can produce big amounts. A second and slightly more subtle but well-known mechanism of varietal differentiation is by means of specific precursors, such as glycosidic or cysteinyl precursors. In this case, the must itself does not display particular varietal characteristics, but these are revealed by fermentation or during maturation. This is the case of aromatic mercaptans, nor-isoprenoids and volatile phenols and vanillins.

However, there is a third and more subtle mechanism which is less well known and much more indirect. The key issue in this case is that the profile of important grape components (only secondarily related to aroma), such as amino acids or fatty acids, is genetically controlled to a great extent. These compounds are the basic building blocks used by yeast to make proteins and membranes, and this means that the yeast will find a different supply of building blocks depending on the variety of must in which it grows. Whatever the supply is, the same proteins and membranes have to be built. As some important wine aromas are just the by-products of the process of protein or membrane building, the profile of such by-products is indirectly controlled by the profile of the supply, which in turn is controlled by the specific genome of the variety of grape.

As expected, this latter mechanism is quantitatively the most important in the differentiation of wines made with neutral varieties. This effect can be seen in Fig. 2a and Fig. 2b. The spider webs shown in such figures make it possible to compare the quantitative content in important wine aromas between monovarietal young Spanish red

wines¹⁰. The aromas of these wines are just slightly different, and not all tasters are able to correctly assign the varietal origin.

FIGURES 2A AND 2B. Spider webs showing significant quantitative differences between monovarietal wines

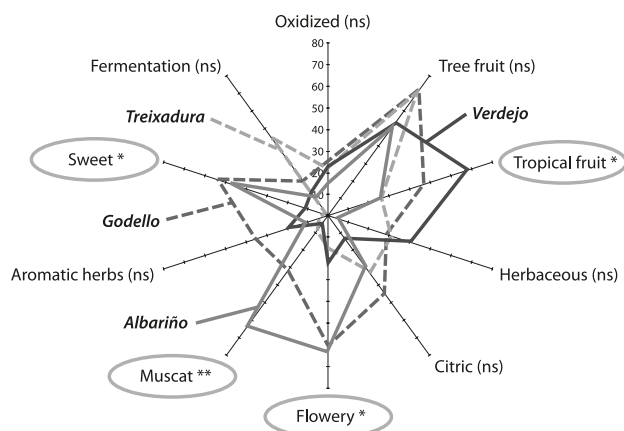


The figures show that the most important differences between wines made from those neutral varieties are due primarily to compounds linked to yeast amino acid metabolism: fusel alcohols, fusel alcohol acetates, isoacids and isoacid ethyl esters. Also remarkable is the presence of some compounds linked to the grape unsaturated fatty acids (γ -nonalactone, c-3-hexenol and hexanol). Although in this overview it is not possible to go further on this issue, it is noteworthy that the aroma profile of a wine, i.e., the relative proportions of aromas it has, is a perfect criterion to classify wines by varietal origin¹¹, far better than the absolute quantitative composition. Since the qualitative characteristics of the aroma perception are more related to the aroma profile than to the absolute amounts, this means that important qualitative characteristics are related to the variety of grape.

In some other cases, the varietal differences between wines become obvious. In a recent experiment⁶, we compared different white wines made with Spanish varieties. The wines were studied by sensory analysis, gas chromatography-olfactometry (GC-O) and by chemical

quantitative analysis. In this case the varietal differences were remarkable and affected, according to the descriptive sensory analysis, the floral, sweet, muscat and tropical fruit notes. The variety known as Verdejo was richest in tropical fruit, while the variety Albariño was richest in muscat, as shown in Fig. 3.

FIGURE 3. Sensory descriptors and scores of a selection of monovarietal Spanish white wines⁶



The sensory scores for such notes were correlated with the GC-O scores obtained using a statistical approach known as Partial Least Square regression (PLSr) and some simple but highly satisfactory models could be built. As an example, the models for the tropical fruit and muscat descriptors are shown below (models for sweet and flowery are similar to the model for muscat).

$$\text{Tropical fruit} = 0.84 I_{\text{mercaptohexylacetate}} - 0.37 I_{\text{methoxypyrazines}} - 0.41 I_{\text{unknown}}^1$$

$$\text{Muscat} = 0.53 I_{\text{linalool}} + 0.39 I_{\text{unknown}}^2 - 0.56 I_{\text{mercaptohexyl acetate}} - 0.51 I_{\text{acetic acid}}$$

It can be observed that the models have a complementary structure as regards to the role played by 3-mercaptohexyl acetate. It is a positive contributor to the tropical fruit note, but it seems to cause the intensity of the muscat note to decrease. That is to say, the notes seem to be competitive. This seems to be quite usual in wine aroma^{5, 12}.

To further check if such models were real or had been obtained just by chance, the models were validated by studying the sensory properties of wines and solutions containing increasing amounts of the compounds taking part in the models. The results of the validations fulfilled completely the suggestions made by the models which allow us to propose the following conclusions:

- In these wines the variety of grape exerts a deep, clear and easily identifiable sensory influence.

- Such sensory influence is mainly related to the quotient linalool/3-mercaptohexyl acetate (both components are impact compounds).
- Methoxypyrazines appear as negative factors as long as their presence is not directly related to any sensory descriptor, but is inversely related to the tropical fruit character.
- Phenylethyl acetate, reinforced by other acetates, can also be a positive contributor to the sweet and floral notes (in this case acting as a family).
- Acetic acid seems to be another negative factor as long as it is negatively correlated to the muscat note and it does not keep any positive relationship with any other sensory note.

Of course, we do not mean that such models can be generalized to other wines and varieties, but we do think that the general structure they have is going to be followed in most cases:

- Whenever compounds (or families) classified as potentially impacting are present at a sufficient level in a wine, they are going to dominate the sensory profile.
- If different impact compounds are present, very likely they will establish a competitive relationship between their corresponding aroma nuances.
- The presence of relatively large amounts of other odorants, such as methoxypyrazines, will probably bring about only a decrease in the odour intensities of some of the odour nuances.

If the list with the most important odorants of wine and the list with the odorants whose level seems to be related to the variety of grape are compared, we will find a nearly complete fit, as it is shown in Table 1. Nearly all the compounds or families classified as impact compounds, with the exceptions of E-whiskylactone (which comes from the wood) and the family of isoaldehydes (which come from the microbiological oxidation of fusel alcohols), have been described in research to be related to the variety of grape.

The role of grape amino acids in varietal aroma

As stated earlier, wines made from different grape varieties usually have different levels of the odorants related to the biosynthesis of amino acids by yeast. This fact led us to think that it was probably caused by the amino acid profile of grape, which, after all, is responsible for, or at least deeply related to, the aroma profile of wine. This hypothesis was confirmed in an experiment where synthetic musts containing amino acid profiles imitating

TABLE 1. A summary of the role played by the variety of grape or the strain of yeast on the levels in wine of the most important odorants of wine (off-flavours excluded)

*1 refers to the existence in the must of important amounts of the free compound.

2a refers to the existence of glycosidic precursors.

2b refers to the existence of cysteinyl precursors.

c refers to the absence of a specific precursor but to the fact that the compound is synthesized "de novo" by the yeast.

Compound	Role of grape variety	Source in grape*	Effect of yeast
Linalool	Very strong	1 and 2a	Yes
Cis-rose oxide	Very strong	Probably 1 and 2a	Unknown
4-methyl-4-mercaptopentan-2-one	Very strong	2b	Yes
3-mercaptohexanol	Very strong	2b	Yes
3-mercaptohexyl acetate	Very strong	c-2b	Yes
Isoamyl acetate	Strong	c	Yes
(E)-whisky lactone	Null	-	No
Diacetyl	Average	c	Unclear
Sotolon	Unknown	Probably c	Unknown
γ -lactones	Average	Probably c	Unclear
Volatile phenols	Strong	2a	Yes
Vanillin and related compounds	Strong	2a	Yes
Burnt sugar compounds	Probably strong	Unknown	Unknown
Fusel alcohol acetates	Strong	c	Yes
Fatty acid ethyl esters	Null	-	Yes
Fino aldehydes	Probably null	-	Unknown
Phenylacetaldehyde	Unknown	c	Yes
Methional	Unknown	c	Unknown
Fusel alcohols	Average	c	Yes
Isoacids	Average	c	Yes
Isoacid ethyl esters	Average	c	Yes
Vinyl phenols	Weak	c	Yes
β -damascenone	Average	2a	Yes

those found in natural musts from different grape varieties were fermented and the aroma compounds formed were similar to the wines analyzed. The results⁷ fulfilled most of the hypothesis and demonstrated that, effectively, the amino acid profile of a must exerts a deep influence on wine aroma. A summary of the findings is listed below:

- The levels of 17 different volatiles (out of a total of 28 analyzed compounds) were significantly affected by the amino acid profile.
- Compounds affected included ethanol and acetic acid, fusel alcohols, fusel alcohols and their acetates, iso acids and their ethyl esters.
- There was a satisfactory correlation between expected and observed aroma profiles. For instance, the "wine" obtained by fermenting an amino acid solution resembling that of a Tempranillo must was found to have the highest levels of isoamyl and β -phenylethyl acetate (see Fig. 2a).
- The relationship between the amino acid profile and the aroma profile was quite complex and multivariate, and in most cases not obvious. For instance, there was not a direct correlation between isoleucine and isoamyl alcohol, although such a correlation existed between β -phenylethanol and phenyl alanine.
- Additional experiments carried out with different yeasts and nitrogen levels indicate in published⁸ and unpublished research that the behaviour is very complex and multivariate (for instance, at a certain level of phenyl alanine, the correlation between this amino acid and phenyl ethanol disappears). This indicates that the final result will depend on the amino acid profile, the total nitrogen available and the nitrogen requirements of yeast.

The role of yeast in varietal aroma

First, we will summarize the compounds or families of compounds whose level in wine has been found to depend on the strain of yeast used (unpublished reports or references^{13, 17}). A summary can be seen in the last column of Table 1. As it can be seen, the strain of yeast exerts a documented effect on many important odorants classified as relevant to the varietal aroma. In some cases there are just not enough data to extract a clear conclusion, since it seems that microbiologists are reluctant to exploit the benefits of analytical chemistry. For instance, it has been documented that cis-rose oxide is the key odour compound characteristic to Gewürztraminer wines^{1, 18}, however, we have not found any report on the influence of the strain of yeast on its synthesis or release.

Not surprisingly, the strain of yeast exerts an important influence on the compounds related to the synthesis of amino acids, such as fusel alcohols and their acetates, isoacids and their ethyl esters. However, data in the table also report for the first time that the level of phenylacetaldehyde can be also significantly influenced by the strain of yeast. This compound is an important oxidation-related

odorant whose synthesis, paralleling that of methional, is probably related to the levels of amino acids remaining in the wine after the fermentation¹⁹. This finding raises a series of important questions, such as if the levels of other related important wine odorants (sotolon and methional), also depend on the strain of yeast, which would mean that the shelf life and future maturation of wine aroma is also related to the strain of yeast.

According to our experience, there are some properties that seem to be quite infrequent among yeast strains. For instance, only one in six yeast strains seems to have a special ability to release additional levels of β -damascenone or to release cysteinyl precursors.

In short, when different yeast strains are compared, most often we will find differences in the levels of the following aroma compounds in wine:

- Isoacids
- Isoacid ethyl esters
- Fusel alcohols
- Fusel alcohol acetates
- Fatty acids and fatty acid ethyl esters
- Vinyl phenols
- Volatile phenols (guaiacol, eugenol, etc.)
- Linalool and other terpenols
- 3-mercaptohexyl acetate
- And only sometimes will it be possible to find differences in β -damascenone, 4-methyl-4-mercaptopentane or 3-mercaptohexanol.

In this last section a series of observations, hypotheses and thoughts on the biotechnological processes required from yeast to form or release the aroma compounds in wine will be presented, organized by groups of aroma compounds in wine.

Fig. 4 summarizes the processes required to form the important cysteinyl derived mercaptans. Precursors of these odorants are amino acids and small peptides and have to be literally digested by yeast to release the odorants. Therefore we can hypothesize that apart from the required β -lyase activity to break the S-bond, the level of the odorants released will depend on the protease activity and on the existence of specific transport systems into the cell. This would imply that the uptake would depend on environmental conditions, particularly on the grape amino acid profile, and on the N/S ratio. Another interesting and important question is the fact that 3-mercaptohexyl acetate, which is the aroma with most pleasant characteristics and has been shown to determine the varietal aroma

nuances of some white wines⁶, does not have a specific precursor in wine, but has been seen to be formed by the acetylation of 3-mercaptohexanol. This process, probably, will be intracellular and will require a strong acyl transferase activity by yeast. In fact, we have empirically found (unpublished report) that the level of this compound is related to the presence of 3-mercaptohexanol and to the presence of isoamyl acetate.

FIGURE 4. Mechanisms presumably involved in the formation of some important wine mercaptans

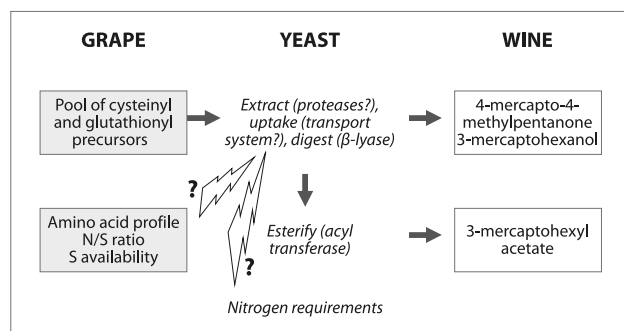
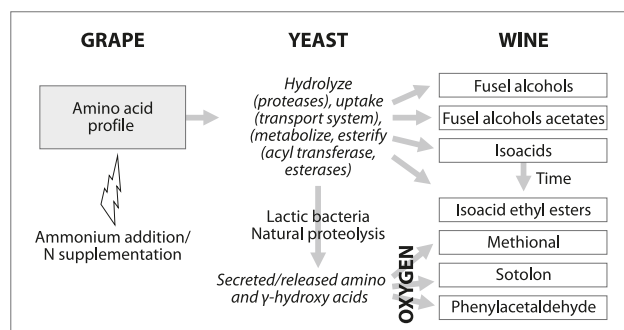


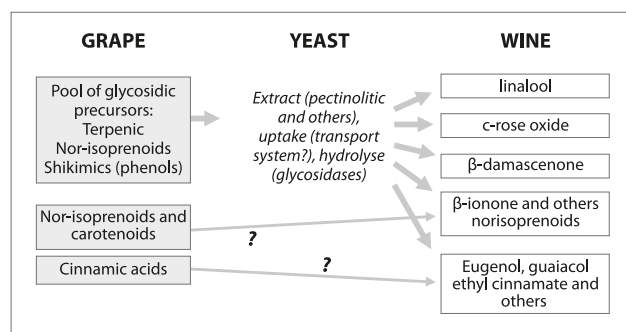
Fig. 5 shows the interrelations between the materials in grapes and wine and the biotechnological processes of yeast for the aroma compounds related to amino acids. As it has been demonstrated, the levels in wine of fusel alcohols, their acetates and of isoacids, are related to the amino acid profile of wine, but are also strongly dependent on the specificities of the nitrogen metabolism of such strain of yeast. Such metabolism must be very complex, since at a first glance it can involve proteases, specific transport systems, the whole pool of enzymes required to synthesize amino acids, plus different esterase activities. In addition, time is an additional factor in the slow esterification of isoacids (in this case the ester is slowly formed by aging), as we speculated before. Yet, it is still necessary to consider that the final amino acid (or γ -hydroxy acid) profile remaining after the fermentation, which can be logically deeply altered by the lactic bacteria and by natural proteolytic processes, will be the source of important odorants formed along the maturation process: methional, sotolon and phenylacetaldehyde.

FIGURE 5. Mechanisms presumably involved in the formation of wine amino acid-related odorants



The relationship between the grape content in glycosidic precursors and the wine content in some aromas is summarized in Fig. 6. In this case, there is a pool of glycosides, another of nor-isoprenoids and carotenoids, and a third one of cinnamic acids. Only the importance of the pool of glycosidic precursors is well documented^{20, 21, 22} at present, and the role of the other precursors remains unclear, although it can be thought that they play some role. Again, the process of release is not straightforward, but different hydrolytic activities can be involved. It can also be thought that part of the hydrolysis take place in the cell, which may imply the presence of a specific transport system.

FIGURE 6. Mechanisms presumably involved in the formation of some odorants from different kinds of precursors in grapes



It must be acknowledged that we know almost nothing about the fate of some other odorants related to unsaturated fatty acids in grapes or about furaneol and other compounds with burnt sugar notes.

Properties of yeast related to wine aroma

In conclusion, it has been demonstrated, or at least there are enough grounds to hypothesize, that the following properties of yeast have a strong influence on the (positive) aroma characteristics of wine:

- The yeast nitrogen (and sulphur) requirements and specific response to the must amino acid profile
- The yeast acyl transferase activity
- The capacity to extract, metabolize and release cysteinyl-related mercaptans
- The yeast capacity to extract and hydrolyze glycosidic precursors
- The amount and profile of nitrogen material released by yeast after fermentation
- The ability to metabolize the grape unsaturated fatty acids and their derivatives
- The ability to release or produce compounds with burnt sugar notes

All these facts and hypothesis reveal what is obvious:

- That wine is produced in a biotechnological process in which a definite number of changes have to take place in order to obtain an adequate yield in aroma compounds.
- That a large part of such changes are carried out by yeast, and that little is known about the biodiversity of yeasts as regards to their ability to form and release aroma compounds.
- That there is enough knowledge, however, to better understand the biodiversity of yeast and to develop rational criteria for selecting (and managing) yeasts able to produce better, richer and more diverse wines expressing the full potential of a given grape.

Acknowledgements

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USE OF YEASTS IN BURGUNDY AND IN OTHER REGIONS: FERMENTATION AND AGING ON LEES

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Introduction

Yeast addition is a very old practice in Burgundy. In the 1930s, a private laboratory grew yeasts on malt following Professor Jacquemin's method and prepared yeast starters with yeast strains from Burgundy's best *terroirs*: Gevrey-Chambertin, Meursault, Vosne-Romanée, Puligny-Montrachet, etc. Unfortunately, the identity of these strains was not controlled and nobody knows what strains of yeast were obtained. Clearly, there was concern about diversifying the strains.

In the mid-1970s, the distribution of winemaking yeasts in the form of active dry biomass increased the use of selected yeasts in the various French wine areas. Only a few strains of *Saccharomyces cerevisiae* were actually dried, and they were standard wine yeasts selected essentially for their good fermentation capacity (short lag phase) and their complete and steady fermentation kinetic. In Burgundy, resistances to ethanol and to low pH were frequently the principal selection criteria. The Université de Bourgogne studied a new method of determining ethanol tolerance in vinification yeasts (Juroszek et al. 1987) and used it thereafter for yeast selection.

Yeast selection and aroma profiles of wines

In the 1990s, the main French wine regions (Bordeaux, Bourgogne, Côtes du Rhône, etc.), for fear of standardization by the use of the same yeast strains, undertook selection processes in their own *terroirs*. We thus returned to the notion of "*levures de cru*" with well-adapted strains, more specifically for revealing the aromas of local grape varieties.

No scientific proof ever came to support the popular belief that superior strains associated with specific vineyards give a distinctive style and complexity to wine. However when the varietal aromas and their precursors are known for a grape variety such as Sauvignon, it is easier to select a specific yeast strain, like VL3, to release aroma from their precursors (Masneuf et al. 1999). When varietal aroma and their precursors are little known or not known at all, as it is the case for Chardonnay and Pinot noir, it is more difficult to select specific yeast strains. The Université de Bourgogne and the Bureau Interprofessionnel des Vins de Bourgogne conducted a yeast selection process that took seven years, between 1987 and 1993.

Table 1 gives a diagram of yeast selection. Yeast samples are collected in musts at the beginning of alcoholic fermentation and on cellar equipment in the best wineries of a particular area. Only a very small number of the thousands of samples collected over a two-to-three-year period are eventually selected as the best ones.

TABLE 1. Yeast selection

Selection of a <i>terroir</i>, area or site
Sampling from grapes and cellars
500-1000 microorganisms
Selection of 1 to 5 strains with good fermentation qualities, aroma/flavour profile, other criteria
Fermentation and drying trials
Sample trials in specific areas

It takes several years of research and experimentation to verify whether the strains have already been isolated and to evaluate their possible impact on the sensory profile

of wine. This work can be conducted only in the laboratory under carefully controlled conditions, but it is only in winery conditions, year after year, that yeast can be fully understood.

The strain CY 3079, for instance, was selected for Chardonnay because of the typicity of the wines obtained after alcoholic and malolactic fermentation, but the final result depends on winemaking conditions: whether it is aged on lees or not. Indeed, the winemaking process itself is always important.

In Burgundy, as shown by Feuillat (1997), a prefermentative maceration at low temperature (8° to 10°C) during a period of three to five days gives more varietal aromas to Pinot noir wines (strawberry, raspberry, blackcurrant, etc.). This phenomenon can be either an enzymatic mechanism or the result of the growth of cryotolerant yeast strains.

Massoutier et al. (1998) showed that, after a few days of growth of an indigenous flora at low temperature, cryotolerant strains of *Saccharomyces* become dominant. These strains belong to the *Saccharomyces bayanus*, *uvarum* variety which is melibiose (+) and whose electrophoretic karyotypes present two specific bands, compared to the electrophoretic karyotypes of *Saccharomyces cerevisiae*.

Inoculations of Pinot noir musts by *S. uvarum* strains were made and their implantation was controlled at different stages of the alcoholic fermentation, at 10° and 25°C. The wines obtained, compared to those made with mesophile strains from *S. cerevisiae* in the same conditions, yield more glycerol at 10°C as well as at 25°C and less ethanol at 25°C (Table 2)

TABLE 2. Production of different compounds at 10°C and 25°C with a mesophilic yeast (*S. cerevisiae*) and with a cryotolerant yeast (*S. uvarum*) during alcoholic fermentation of a grape must

	Ethanol (%)	Glycerol (g/L)	Isoamyl alcohol (mg/L)*	2-phenyl ethyl alcohol (mg/L)
<i>S. cerevisiae</i>				
25°C	12.8	7.4	146 ± 12	45 ± 4
10°C	8.7	5.1	179 ± 15	47 ± 5
<i>S. uvarum</i>				
25°C	11.8	8.2	280 ± 30	216 ± 23
10°C	10.8	6.3	265 ± 28	225 ± 25

The fermentation tests were carried out in triplicate. Among volatile compounds, the most significant difference was observed for isoamyl alcohol and for 2-phenyl ethyl alcohol. Isoamyl alcohol was produced by *S. uvarum* at levels nearly twice as high as those produced by *S.*

cerevisiae. The difference is still higher for 2-phenyl ethanol. *S. uvarum* produces more than four times as much as *S. cerevisiae* at both low and intermediate temperatures.

The compound 2-phenyl ethanol (sensory threshold: 34 mg/L) has a pleasant rose-like odour and at low level may be regarded as a positive component. However, the large amounts produced by *S. uvarum* could affect wine quality in a negative way. Sensory analysis shows that the addition of 150 mg/L of 2-phenyl ethanol increases floral descriptors (rose, honeysuckle, violet), but hides fruit aroma (cherry, blackcurrant, raspberry) which are the main Pinot noir varietal aromas. These results agree with those of Vila (1998).

Yeast selection and colloid release: Influence of yeast glycosylated proteins on polyphenols

Yeast glucans and glycosylated proteins present in the cell wall are released:

- Partly during alcoholic fermentation by budding living yeasts
- Partly after fermentation during autolysis by dead cells.

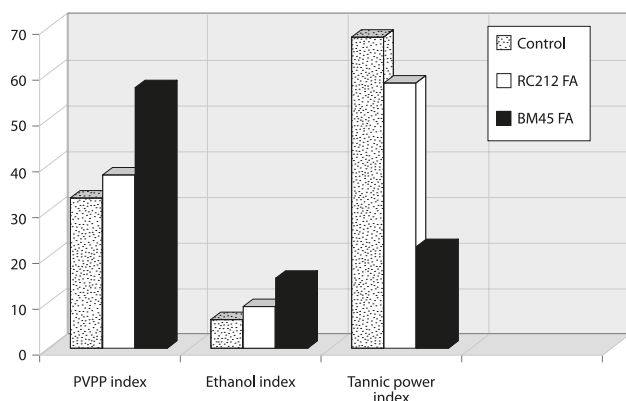
Yeast macromolecules, and especially yeast glycosylated proteins, play several oenological roles that are now very well known: activation of malolactic bacteria, improvement of wine stability (glycosylated proteins prevent haze formation and potassium hydrogenotartrate crystallisation), interaction with aroma compounds, improved quality of foam for sparkling wines, etc.

More recently, Saucier et al. (2000) studied the interaction of polysaccharides with phenolic compounds in red wines.

In Burgundy, red wines are increasingly being aged on lees, sometimes with the addition of exogenous glucanase preparations, in order to bring more suppleness and to reduce the quantity of astringent tannins (Feuillat et al. 2001). The influence of glycosylated proteins on tannin properties depends on the yeast strain, and the glycosylated proteins released during alcoholic fermentation seem more reactive than those released during autolysis (Escot et al. 2001).

Wine tannins are less astringent with the addition of glycosylated proteins, as shown by the decrease of the gelatine index. The polymerization of tannins together and their association with polysaccharides are shown by the increase of the PVPP and ethanol indices (Fig. 1).

FIGURE 1. Influence of glycosylated proteins (100 mg/L) released by *S. cerevisiae* BM45 and RC212 on Pinot noir tannin properties



Glycosylated proteins from *S. cerevisiae* BM45 are more reactive than those from *S. cerevisiae* RC212. We know (Rosi et al. 2000) that BM45 is a high producer of macromolecules during alcoholic fermentation and that RC212 is a lower producer. The main composition difference between the BM45 and RC212 glycosylated proteins is the mannose/glucose ratio. This ratio is nearly 1 for BM45 glycosylated proteins, whereas mannose is the main component (80%) of macromolecules from the RC212 strain and glucose represents only 10%.

More recently, Escot (2003) and Charpentier et al. (2004) studied the influence of glycosylated yeast proteins on tannin aggregation in a model wine solution, using a spectrophotometric method (absorbance 700 nm). A stability coefficient (SC) was defined as the ratio between the absorbance of tannins alone and the absorbance of tannins with glycosylated proteins.

$$SC = \frac{Abs. 700\ nm\ (Tannins)}{Abs. 700\ nm\ (Tannins + glycosylated\ proteins)}$$

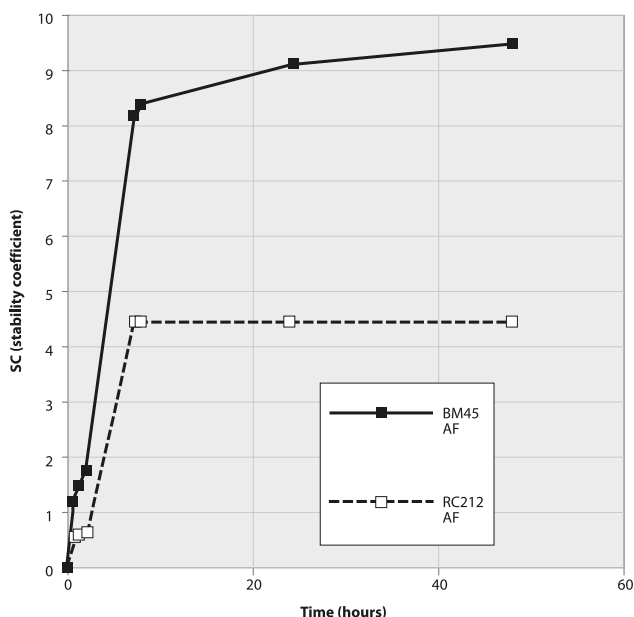
After the first measurement, samples were stored at study temperature and absorbance was measured every hour after shaking during the first 24 hours, then every day during the following four days. When the SC is superior to one, tannin aggregation is less important. In the presence of glycosylated proteins from BM45 released during alcoholic fermentation, the stabilization of tannins is nine times more important than that of tannins alone, and twice as high as in the presence of glycosylated proteins from RC212 (Fig. 2).

Results found for tannin aggregation with glycosylated proteins are in keeping with the findings described previously.

The study of the degree of the effect of polymerization (mDP) of tannins (ranging from 3.6 to 10.0) on tannin ag-

gregation showed that glycosylated proteins seem to interact with tannins of high molecular size (mDP 10.0 fraction). This result agrees with that of Riou et al. (2001) who observed that tannins with mDP (6.9) can form aggregates liable to interact with macromolecules and/or surface. Glycosylated proteins, and especially glycosylated proteins BM45 released during alcoholic fermentation, seem to coat tannins, thereby preventing their precipitation, which leads to improved colour stability and decreased astringency.

FIGURE 2. Influence on tannin stability coefficient of glycosylated proteins (1 g/L) from *S. cerevisiae* BM45 and RC212 yeast strains released during alcoholic fermentation



To specify the nature of the binding involved in glycosylated proteins and tannin interaction, charge densities and a conformational study should be investigated.

Yeast selection and peptide release during autolysis

Two main enzymatic mechanisms lead the autolysis of dead yeasts:

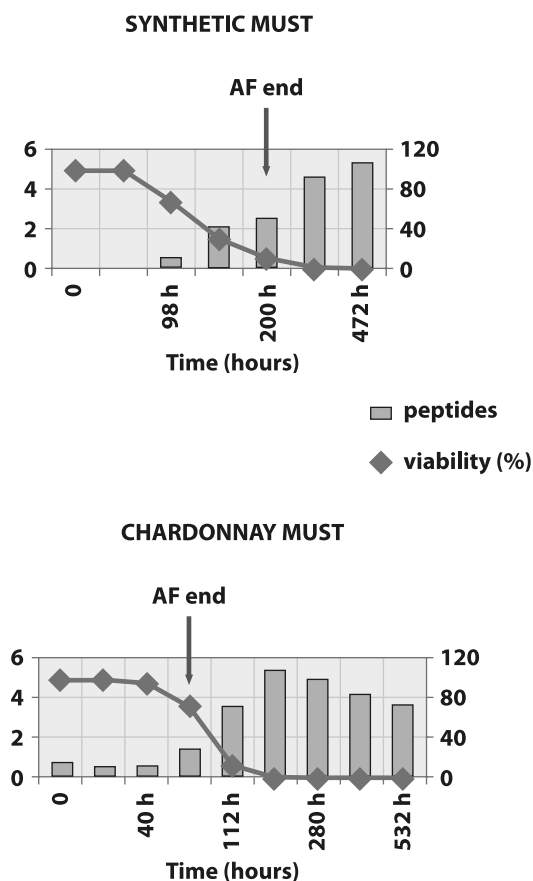
- Cell wall degradation by β -glucanases and release of glucans and glycosylated proteins
- Intracellular proteins degradation by proteases and release of amino acids and peptides.

Using pepstatine as a specific inhibitor, Lurton et al. (1989) demonstrated the key role of protease A in autolysis in an acidic pH. It acts as an endoprotease because most of the liberated nitrogen (about 60%) consists of peptides of low molecular weight (between 0.7 and 5 Kda).

Wines are 10 to 15 times richer in peptides than corresponding musts (Carneville et al. 1999). Peptides are re-

leased at the end of alcoholic fermentation when yeasts enter the stationary phase of growth and when cell viability decreases from 80% to 50%. Peptide production increases drastically when they are no viable cells and when the medium temperature is held to 35°C to accelerate autolysis. The same kinetic of peptide release is found in synthetic must and in grape must (Fig. 3).

FIGURE 3. Evolution of peptides (mmoles/L) during alcoholic fermentation and the first hours of autolysis

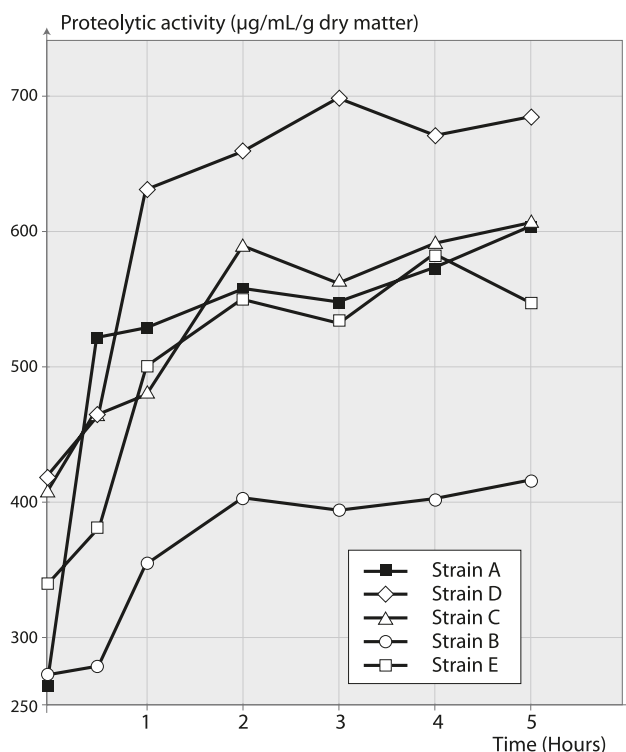


The amino acid composition of peptides is not the same during alcoholic fermentation and during autolysis (Alexandre et al. 2001, Perrot et al. 2002). After 54 days of autolysis in synthetic wine (pH 3.5), we observed a decrease of Asx and Glx (about 50%), as well as of Ser and Val while an enrichment in Lys was noted. These results showed that, depending on aging time on yeast lees, peptide composition could vary, leading to peptides of different sensory properties, such as sweetness or bitterness. It should be noted that Phe-, Tyr- and Leu-containing peptides seemed to represent a minor fraction of peptides released during autolysis, which is important since it has been shown that such peptides were responsible for bitter taste.

The sensory properties of peptides were studied in Champagne wines (Desportes et al. 1999) but the sensory thresholds of the identified peptides were not reached,

although there might be some synergy effects. The enrichment of wine with peptides can be made with addition of yeast autolysates. One must choose a highly proteolytic yeast strain to prepare them. Indeed, intracellular proteolytic activity once cells are dead is highly variable from one strain to another (Fig. 4).

FIGURE 4. Intracellular proteolytic activity of different *Saccharomyces cerevisiae* strains at the end of alcoholic fermentation



Autolysis was carried out in model wine at 50°C during variable periods of time, in order to obtain a more or less high protein degradation. Cellular hulls are eliminated and the supernatant is dried by heat. These preparations are rich in amino residues (short peptides), proteins and sugar. Added to wine at 30 to 50 g/hL, some autolysates give rounder and fuller wines (Table 3). The physiological state of yeasts seems to strongly influence the autolysate properties. For sparkling wines, the very same autolysates could give a thinner and more persistent foam. Mellowness and sweetness in wines increase as during a long aging on lees.

TABLE 3. Influence of the addition of yeast autolysates (0.5 g/L) on the sensory properties of Champagne wine after a 24-month aging

Samples (Pairs)	Preferences	Flavour descriptor
Control	4	More acid
Wine + Autolysate A	10	More maturity
Control	11	More acid
Wine + Autolysate B	4	Slightly oxidized

Autolysate A is obtained from yeasts at the end of alcoholic fermentation

Autolysate B is obtained from dry yeasts

Conclusion

Today, more than 200 oenological yeast strains have been isolated and selected. New findings, such as the identification of varietal aromas and their precursors that were little known or unknown for some varieties, or the setting up of very specific vinification processes such as pre-fermentation maceration at low temperature, justify new yeast selections.

In recent years, the attention of selectors was drawn to the metabolites released by yeast either during alcoholic fermentation or during autolysis, in the case of aging on lees. The release of glycosylated proteins from yeast strains is highly variable from one strain to another but the oenological roles of these compounds are as multiple as they are interesting. Other metabolites, such as peptides or nucleotides, which are flavour agents, are currently being studied. Nevertheless, these metabolites are often present in wine under their sensory thresholds, hence the selection of new yeast strains for the preparation of yeast additives rich in these compounds.

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OUTLOOK OF THE OIV ON THE USE OF BIOLOGICAL TOOLS IN WINES

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The International Organisation of Vine and Wine (OIV), is an intergovernmental organization responsible for laying the foundations for the harmonization of production techniques between countries, through the scientific and technical activities carried out by the different groups to which it is linked.

The work these groups carry out is the result of a series of decisions taken by the OIV Experts' Groups and its ruling bodies. The decisions are taken by consensus, meaning that its members (the member states) must adopt common positions.

The decisions of each of the member states are presented, taking into account the opinion of the different agents in the production and marketing chain of the winemaking sector (via their associations), of consumers, as well as the opinions of scientific and technical bodies working for the sector. The position of a certain member state may be made with regard to a specific problem and is therefore the result of agreement within its sector and the understanding of the quality and food safety assurances required by consumers. This position is presented through an official state-appointed delegate to the pertinent authorities in the OIV.

The OIV is structured as follows:

- General Assembly
- Steering Committee
- Scientific and Technical Committee
- Chairman
- Deputy Chairmen
- Executive Committee

- Commissions, Sub-commissions and Experts' Groups
- Secretariat, managed by the General Director

Within this structure, it is the "Wine Microbiology" Experts' Group, part of the Oenology Commission and answering to the Scientific and Technical Committee, that is entrusted with the responsibility for wine microbiology or any other viti-, vinicultural product. It is at this level that a position taking into account solely scientific or technical considerations must be put forward to the Steering Committee and to the General Assembly. These two bodies will, where appropriate, introduce amendments to it, bearing in mind other considerations, suspend or approve it and it is voted in any event in the General Assembly.

Consequences of the work of the OIV

At this point, it is useful to consider what role the OIV plays in this regard and the consequences/implications vis-à-vis other legislative bodies of the member states. As far as the latter question is concerned, the OIV is the main source of ideas or the reference point in the regulations implemented in numerous countries, mainly in the European Union and its immediate neighbours. This does not imply that these countries have to observe the opinions or suggestions of the OIV, given that its decisions adopt usages which are accepted or acknowledged as appropriate for winemaking by all member states, without needing to be incorporated in the regulations of each country.

As a result of its scientific and technical activity, the OIV has a set of rules and regulations which is included in different documents and which can be freely accessed

on the Website www.oiv.int, in the Publications Section. These documents are:

- the International Code of Oenological Practices
- the International Oenological Codex, and
- the Compendium of International Methods of Analysis of Wine and Musts.

In the first, the **Code**, information is provided on the objectives and the scope of a particular practice (products, materials, etc.), on whether said practice is authorized and the conditions for its use. The **Codex** provides information on the characteristics applicable to the products or materials which have been authorized in the Code. Lastly, the **Compendium** of methods of analysis concerns the analytical systems for ascertaining the presence of products or materials which may remain as the result of a certain practice.

As far as the issues of microbiology, and, in a broader sense, the questions of oenological biotechnology are concerned, it is the "Wine Microbiology" Experts' Group that submits documents to the General Assembly for approval as Resolutions of the Assembly and which are introduced in the Code of Oenological Practices. If materials and/or technical analyses are subsequently involved, it is then the responsibility of the Methods of Analysis Sub-commission to establish the pertinent characteristics and analysis methods.

Examples of said documents are those relating to the question of Microbiological acidification (ENO Resolution 5/2003), specifically with regard to *Saccharomyces* yeast (ENO Resolution 4/2002); and Microbiological de-acidification (see Appendix 1), including Resolutions ENO 3/2003 (general), ENO 5/2002 and ENO 1/2003, addressing the possibility of performing said de-acidification with *Saccharomyces* and *Schizosaccharomyces* type yeasts, respectively.

These are ways of responding to acidification or de-acidification requirements without resorting to corrective practices which do not form part of the working methods of certain "bio or ecological" type wineries or even of traditional wineries, which wish to keep the use of additives to a minimum.

Each one of these resolutions underlines that the materials which are used for the working practices (in the case of microbiological practices, yeast and bacteria) must "conform to the requirements of the International Oenological Codex," to which we referred previously. The Codex is therefore the document which analyzes the nature of the material to be used, its origin, purity, information condi-

tions and in general all those conditions which may be of interest to the user or the manufacturer.

The current outlook of the OIV on certain matters in the sphere of oenological microbiology is the result of the working dynamics followed so far and of the current reflection carried out in the Organisation. This will be briefly addressed before moving on to the issues which are the title of the talk.

OIV Guidelines

The OIV has drawn up and practically approved the Strategic Plan up to 2008. It will be approved by the General Assembly that will meet in October 2005. The Plan contains what are still proposals (as of the date of this statement) regarding the questions to be addressed. The most important as far as microbiology is concerned are the following:

- Food safety: Evaluate health risks and give food safety recommendations on the toxic compounds, including those produced by microorganisms of oenological interest.
- Biodiversity:
 - Establish systems or mechanisms for the preservation and exchange of microorganisms.
 - Study of the diverse relationships which can be established between the variety of microbiological species (antagonism, mutualism, symbiosis) in the vine-vineyard ecosystem.
 - Propose molecular techniques for identifying and characterizing the different microbial species.
- Innovations in biotechnology:
 - Make a summary of the current world situation of GMOs in the wine sector.
 - Evaluate the environmental, agricultural (cultural practices) and economic implications and impact of GMOs in the production and consumption sectors.
 - Compile "Genome map" data.
 - Promote research on genome analysis and genetic expression mechanisms.
- Methods of analysis:
 - Prepare and harmonize methods of analysis in microbiology.
 - Prepare qualitative specifications of oenological products (including biological materials).

It is evident that there are currently three major questions about which there has been much debate:

- Food safety, where there is discussion on the presence of biogenic amines (mainly those that can give rise to allergy problems or could strengthen the action of allergies), arising from the action of both lactic bacteria (a problem that is well known, but for which a solution has yet to be found) and yeast during alcoholic fermentation.
- The preservation of the biodiversity and the research into new complementarities of oenological interest afforded by different species of yeast and bacteria.
- The taking of a position regarding genetically modified microorganisms (GMOs) used in oenology, mainly yeasts. This subject will be addressed in the following section.

The OIV and GMOs

Mention was made previously of the OIV's sensitivity towards companies and consumers. In this regard, it must also be said that the companies are well aware that most consumers have a high regard for wines made with minimum recourse to technological manipulation.

Some companies are aware that certain consumers do not even understand the use of yeasts or bacteria selected in the manufacturing process, in the same way as some consumers are suspicious when they hear talk of virus-free clones in vines. The degree of rejection of biotechnology associated with GMOs by winemaking companies is in many cases due to a cautious stance, until the passing of time allows the consumer or the market to become used to GMOs, and wine companies realize the positive effects of genetically modified yeasts (GMY).

In this regard, although the OIV is aware of the sensitivity of the vast majority of wineries regarding GMY, it has not ceased to promote research and innovations based on biotechnology. This does not imply that it acts with the speed that certain members of the scientific community would wish for. The OIV is perfectly aware that, at some point in the future, society will accept foods derived from GMY or foods produced with the help of GMYs on the same footing as more conventional foods, and, consequently, that the sector should be in a position to make use of these biotechnological resources if it so wished.

Here we shall not touch upon the new possibilities offered by biotechnology to complement the functions of yeasts. The types of genetic modification that are possible and of interest from the technological standpoint are outlined in other talks in this meeting. It is important to note, however, that genetic modification is not the only way of finding biological material offering these complementarities:

some – not all, because these genic expressions derive from different species and organisms – could be achieved by conventional biotechnology, although in a more uncertain and costly fashion and certainly in the very long term.

The attitude of caution is also evident in the as yet undecided position of the wine-producing countries and leads to the OIV's own position. The organization stands at a point of reflection and search for consensus on different points:

- The risks of dissemination of GMOs in the viti- vinicultural environment
- The possibility that the GMOs might be uncompetitive and self-destructive
- Information for public opinion on the use of GMOs, as far as public health, ethics and education are concerned.

The "Wine Microbiology" Experts' Group has been given the task of providing information to make a decision on the above points. This Group has considered studies with conventional microorganisms – extrapolating the results to GMOs – which demonstrate that dissemination within a winery and in the viti- vinicultural environment is possible but limited. Furthermore, the imposition of the commercial microorganisms on autochthonous flora is also very limited, and the studies do not evidence any type of predominance.

There are also techniques that induce the self-destruction of the microorganism and could be introduced into the genetic code of the GMOs, preventing them from proliferating in the environment. Naturally, this would be of twofold interest: it could appease the most conservative positions, which are suspicious of genetic modification (because the release of GMOs in the environment is prevented), and the commercial interest of the producers of these microorganisms. In any event, via the **Oenological Product Codex**, and provided a consensus between countries is reached, the OIV can ensure that these conditions are inherent in the GMOs offered for trade.

So far, with these two points, there is a scientific basis for the recommendations of the OIV. The question of public information is quite different, however. Here different policies come into play from the ones that regulate viti- vinicultural production, and often have little to do with scientific considerations.

Before a position is taken, it is important to reflect on which products are classified as genetically modified and the obligation of making this known on the label. Would

wine produced with a GMY be included in this category? Let us consider: if the yeast acts solely as an agent during the fermenting process, which is not integrated in the wine, and if the presence of the yeast in that process is limited, and indeed disappears entirely during the final treatments (clearing and filtering), it may be concluded that wine should not be considered to be a genetically modified substance and should not be labelled as such.

In this regard, it should be noted that this same view currently exists in the European Union, made by the Standing Committee on the Food Chain and Animal Health and concerning foods (and feeds) produced by fermentation using GMOs. Two positions would exist: if the food is made **with the help** of a GMO, or if the food is produced **on the basis of** a GMO. If the former position (which seems to be the position preferred by virtually all the countries taking part in the Committee) is decided upon, the use of a GMO and the marketing of wine would cease to be subject to prior administrative authorization and there would be no mandatory requirement to make use of the GMY, provided the materials obtained with the GMY did not exceed 0.9 % of the ingredients of the food, and it could be demonstrated that this presence is “accidental or technically inevitable.”

This position of the EU (where the highest degree of resistance is to be found), could decide the position of the OIV concerning GMOs, given that certain countries, such as the United States, Australia, New Zealand and South Africa, which have been at the forefront of research on GMOs, would be likely to agree with their use.

This possibility does not imply that traceability activities should not be stepped up, both the traceability required by administrative authorities and that of the different agents in the production and distribution chain.

If labelling is not mandatory, GMY will have the acknowledgment that each oenologist and the particular nature of each company wish to give them.

Lastly, we turn to the third subject of this talk.

Biological material for use in winemaking

Or to put it another way: Which biological tools offered to oenologists have received a favourable opinion from the OIV and in what conditions?

The response focuses on the two best-known agents, both of an active nature: the yeast for alcoholic fermentation (AF) and the bacteria for malolactic fermentation (MLF).

Yeast is considered to be inherent to the manufacturing process and is not afforded special treatment in the Code. Yeast is addressed only in the Codex, by means of Resolution 16/2003, which contains the technical specifications of commercial preparations of active dry yeast. The specifications also include the predominant position to date, namely that prior administrative authorization is required to obtaining and using GMY.

The resolution states that the genus and the species must figure in the commercial preparation, taking for granted that any yeast that ferments can be used in AF. However, special mention is made of the *Schizosaccharomyces* yeast strains, which are included in the Code (Resolutions 16/1970 and 4/1980) and in the Codex (Resolution 16/2003). Nor does their use in the EU pose any problems according to Regulation 1493/1999.

The use of lactic bacteria is included in the Code (Resolution 4/1980) and in the Codex (Resolution 15/2003). In the latter it is mentioned that lactic bacteria can belong to the *Oenococcus*, *Lactobacillus* and *Pediococcus* genera. Furthermore, genetically modified bacteria are treated in the same way as yeast.

In addition, oenologists are aware of the existence of other materials – either on the market or in development – which are also options for use. In this group of materials, we find the following products derived from yeasts:

- Inactive products (inert yeasts) and
- Degradation products, inter alia:
 - Yeast walls
 - Autolysed yeasts
 - Mannoproteins.

In the Code, autolysed yeasts, yeast crusts and inert cells are considered to be activators of fermentation (Resolution 7/97).

The yeast crusts (which are described in different texts as yeast walls, cellular casings, enzymatic yeast wall preparations and also as yeast cell wall preparations), are authorized by Resolution 5/88 of the Code and mentioned in Resolution 2/2003 on fermentation walls. Nevertheless, their commercial characteristics are yet to be determined, even though the Codex entry is at a very advanced stage.

Autolysed yeasts and inert cells (or inert yeasts) are mentioned in the Code, although they do not figure as an authorized practice, nor have they been determined via the Codex. A legal vacuum would therefore surround the use of these substances. Autolysed yeasts sometimes appear as yeast extracts or yeast residues, although inert cells could also be considered as residues. Should they be treated in

a different way? Although for the latter the critical issue is whether they can be treated as oenological products (a complicated and drawn-out process) or in the same way as fermentation yeast, albeit without fermenting capacity, with the remaining specifications in the Codex being considered to be valid, in which case their approval could be an easier task.

Mannoproteins are an authorized oenological practice and are included in the Code, in Resolution 4/2001, which has subsequently been corrected and extended for use in all types of wines by Resolution 15/2005. Its prescriptions are set out in the Codex via Resolution 26/2004.

In view of the title of this talk, it is logical to think that the outlook in question can only be official when it is reflected in the Resolutions of the OIV. Consequently, the document before you is simply the view of one person, albeit one with responsibility in the “Wine Microbiology” Experts’ Group of the OIV, who can only provide a personal view of the problems discussed in the organization and the problems that are likely to arise, and explain the actions which have been taken.

WINE YEAST STRAIN DEVELOPMENT STRATEGIES: POSSIBILITIES AND LIMITATIONS

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In recent years, the selection of new and the improvement of existing wine yeast strains has received increased attention in the scientific community. The reasons behind this interest can be found in both science and economics. From a scientific point of view, the development of new technologies and strategies – based on traditional breeding/selection and the advances of molecular biology and global analysis tools (“omics”) – has opened up exciting new and previously unimaginable opportunities, while specific demands by winemakers and increasing competition between yeast producers has generated a sustained demand for new strains. This review will provide an overview of some of the existing technologies to select and improve wine yeast strains, with a particular focus on theoretical and practical limitations.

Introduction

Archaeological evidence shows that winemaking is a biotechnology dating back to at least 5000 B.C., suggesting that the wine yeast *Saccharomyces cerevisiae* can be described as the first domesticated microorganism. While the first winemakers were obviously not aware of the presence of yeast, and clearly did not consciously select specific strains, it is likely that the artificial ecological niche provided by their activities, essentially the regular provision of a significant volume of a high energy substrate in a more or less anaerobic environment, led to evolutionary adaptations that resulted in today's wine yeast.

While there is no direct scientific evidence for this assumption, several lines of evidence suggest that this is indeed the case:

- Data show that it was indeed *S. cerevisiae* that was responsible for ancient winemaking (Cavaliere et al. 2003).
- Very few, if any, of the commercially used wine yeast strains can be found or isolated in vineyards, even after several years of having been used in a specific cellar. This may indicate that wine yeast strains are in fact not well adapted to other “natural” environments (Bauer et al. 2004; Valero et al. 2005).
- The winemaking environment, even in its most simple form, is clearly very different from any natural environment, and provides selective pressures that are significantly different from any naturally occurring ecological niche. Fermenting grape juice is in many regards an extreme environment, and relatively few species have successfully adapted to conducting or just surviving the fermentation process.
- The number of generations required for evolutionary change within *S. cerevisiae* populations when grown in a selective environment has been measured in the laboratory, and it has been shown that such changes do occur on a relatively rapid and regular basis, as long as strong selection pressure is applied (Elena and Lenski, 2003). So-called “natural” wine yeast, even those dominating spontaneous fermentations, is therefore arguably a direct result of human intervention.

What are the selection pressures that apply specifically to winemaking and that require specifically adapted strains? Grape must is a liquid containing a very high concentration of excellent carbon sources, the hexoses glucose and fructose. It becomes reliably available in an annual cycle, and is further characterized by:

- An imbalance between the amount of sugar and the relatively low concentrations of other essential nutrients, in particular nitrogen and some “survival factors” like fatty acids and sterols;
- A relatively low pH (as far as natural environments go, a pH of 3.5 can be considered extreme);
- An environment that quickly becomes anaerobic once biological activity starts to take place.

It is a clear indication of the extreme nature of this environment that today's strain development programs are still focusing on developing strains that can better deal with these basic environmental constraints.

However, oenological strains are selected for more than just a general ability to ferment grape must to dryness (Barre 1993; Dequin 2001; Pretorius 2000, 2002; Pretorius and Bauer 2002). Many new criteria have been added to the list of desirable traits over the past years, and are presented in a summarized form in Table 1. In addition to general fermentative ability, these traits are frequently related to the yeast's contribution to wine quality and nutritional value. Yeast can impart specific aroma profiles or modify the concentration of metabolites that have been associated with beneficial or negative consequences on human health. The traits listed in Table 1 under Fermentation performance can be described as generic (all wine yeast strains should satisfy at least some of the minimal criteria for each of these traits). In addition to those generic traits, the yeast industry is actively encouraging the development of more and more specialized yeast strains with very specific aroma profiles and other characteristics. Furthermore, the trend is towards the development of regional yeast strains that would give expression to the *terroir* of a specific region. From a scientific point of view, the concept of *terroir* is rather controversial, since today's viti- and vinicultural practices by and large can supersede whatever influence the factors defining the *terroir* concept may have had.

Today, most winemakers have a good idea about what type or style of wine they would like to produce to be able to satisfy a specific market. It should be highlighted here that this trend, contrary to the impression of many, does not necessarily lead to a general standardization of wine. Indeed, the quality wine market can best be described as a series of niche markets where diversity is an essential element of the attractiveness of the product. The essential challenge then to any winemaker, whether of bulk or quality wine, is to produce a product that closely meets the expectations of a specific segment of the market. It is generally accepted today that inoculation with specific commercial wine yeast strains can contribute significant-

ly to achieving these aims (Barre et al. 1993; Pretorius 2002).

This situation has resulted in sustained efforts by research groups to search for and develop new wine yeast strains that would be able to impart new and different characteristics to the wine, while being better able to withstand the extreme conditions encountered during the winemaking process. Two elements, one of a scientific nature, the other of economic, give strong impetus to these research efforts: From a scientific point of view, wine yeast strain development benefits from the “model organism” status of *S. cerevisiae*. Indeed, to date, *S. cerevisiae* is probably the most studied and best understood of all organisms. The genome of this yeast was the first eukaryotic genome to be sequenced, and many insights gained by using *S. cerevisiae* as a model have had tremendous influence on our understanding of living systems in general, including significant new developments in the medical sciences. Today, *S. cerevisiae* is leading new developments in the biological sciences in the fields of functional genomics, which refers to the global analysis of organisms. On the economic side, the new demands by winemakers have lead to increased competition between wine yeast strain producers, who are aiming at satisfying the market for specialty yeast.

In this review, the strategies used for strain development are summarized, and their advantages and limitations will be discussed.

Methods for strain development

Several excellent reviews on strain development have been published in the past few years (Barre 1993; Dequin 2001; Pretorius 2002; Pretorius and Bauer 2002). The following section limits itself to some essential aspects that are of importance for assessing the potential of each technology. The methods are briefly summarized in Table 2 (adapted from Pretorius and Bauer 2002).

NATURAL ISOLATES AND VARIANTS

Yeast strains that are able to ferment grape must to dryness are present naturally after pressing and are responsible for spontaneous fermentation. Studies have shown that these strains originate either from the berry or from the winery equipment. Most, but not all of these strains, belong to the species *S. cerevisiae*. There are, however, several other yeast species that are able to ferment grape must to dryness, but in a competitive situation, *S. cerevisiae* is usually able to outgrow all other species and to dominate at least the later stages of fermentation.

Various programs have been carried out to isolate yeast strains that are suitable for commercial use from spontaneous fermentation, and most currently sold strains are the result of such selection programs. Most of these strains are of the species *S. cerevisiae*, but some strains have been shown to be natural hybrids between *S. cerevisiae* and other closely related yeast species (de Barros Lopes et al. 2002). With regard to these taxonomic classifications, it should, however, be kept in mind that with more molecular information becoming available, in particular entire genome sequences, the species concept and the taxonomy of yeast are bound to undergo significant changes in the future.

These “natural” isolates are clearly able to satisfy all the basic requirements of winemaking. They also provide a number of options for winemakers, since each strain displays specific traits and characteristics, making it more or less suitable for specific types and styles of wine. However, and as the demand for new and better strains clearly shows, they clearly offer insufficient variety to satisfy all the technological and quality criteria of winemakers. In particular, when comparing the characteristics of existing wine yeast strains with the list of criteria in Table 1, it becomes clear that no individual strain offers optimal combinations of all the desired traits. This is not surprising, since the strains that occur naturally are those that are best adapted to the winemaking environment from an ecological point of view, i.e., the “fittest” strain in wine-making conditions. It is unlikely that such strains would consider nice-to-haves regarding aroma compound production and other parameters as essential for survival. While it is clearly possible to select the best of the existing strains, and that these strains may have an additional appeal to the market if they have been isolated from the local environment (i.e., *terroir* yeast – although, and as stated above, there is no scientific evidence for such a connection), further optimization of these yeast strains is desirable from a winemaking point of view.

HYBRIDS

The name “hybrid,” as used by yeast producers, is in many ways scientifically misleading. Indeed, by definition, all existing yeast strains are “hybrids” in the sense that they combine the DNA of two parents of different genetic constitutions. However, in the wine yeast industry, the term hybrid refers to yeast that originates from a specific breeding program involving two or more known parental strains, which in some cases can be from different, but always closely related, species (interspecific hybridization).

Various strategies have been used for the generation of hybrid strains and are briefly described in Table 2. All of the hybridization methodologies are random, and the result is never predictable. The easiest and most common method of generating hybrids is to use the natural sexual reproduction of yeast: This involves the sporulation of parental strains, followed by mating of the spores to obtain strains that carry 50% of the DNA of each parent. Whatever method is used, the resulting strains have to be further analyzed to verify their usefulness as potential wine yeast strains. Indeed, the random mixing of parental DNA as it occurs in all hybridization strategies is far more likely to result in strains of lesser winemaking ability than in any specific improvement. The reason for this is simple: wine yeast strains are already pretty good at what they are doing. Since many of the traits that make a yeast a “good” wine yeast are polygenic (are due to the specific combination of many, sometimes hundreds of genes), it is much easier to “destroy” the successful combination found in the parental strains than to generate a strain that shows specific improvements. For this reason, any hybridization strategy has to use specific strategies to select those strains that display the characteristics that correspond to the desired outcome.

Various strategies to achieve such outcomes for industrial yeast strains have been described in the literature (Codon et al. 2003), and include so-called enrichment strategies (selection that enriches a mixed culture for strains that display a specific desired trait), directed evolution (maintaining yeast strains for long periods of time and many generations under strongly selective conditions to force evolutionary adaptation) or mating strategies based on homozygous parental strains displaying desired characteristics (Marullo et al. 2004).

MUTANTS

Mutants, as their name indicates, are mutagenized forms of existing strains. Again, from a scientific point of view, it is not sure that the concept makes much sense at all. Mutations naturally occur all the time and every single yeast cell can be considered as a mutant since it is highly unlikely that the replication of 26 megabases (26 million base pairs, approximately the amount of DNA that is found in a diploid wine yeast), which has to happen during every cell division cycle, proceeds without a single error.

In yeast development programs, mutants are the products of a deliberate strategy to create a large number of DNA mutations by applying a mutagenic agent, of either a physical (UV light) or chemical (various compounds) nature. These agents usually damage DNA, directly or in-

directly, leading to a significant increase in the number of errors made by the DNA replication machinery during cell division. As a result, after applying a mutagenic treatment to a culture of any given strain, the culture will contain as many different strains as there are cells in the culture at the time of mutagenesis. While the application of mutagenesis is technically easy, the problem with the strategy is similar as the one encountered for hybrids: the chance of getting it wrong is significantly higher than the chance of getting it right. Any mutagenesis therefore has to be followed by a selection strategy allowing isolating the relatively few improved strains from the bulk of the rather negatively affected majority of strains.

GENETIC MODIFICATION (GM)

The last method of strain improvement to be discussed in this review is the highly contested method of genetic modification or genetic engineering. GM refers to technologies that use the *in vitro* modification of fragments of DNA, followed by their (re-)implantation into the genome of a target species through a process known as transformation. Regarding the use of GM technology for the improvement of wine yeast strains, it has to be mentioned that *S. cerevisiae* has over the past three decades served as one of the major scientific model systems to provide a better understanding of the internal working of a living cell. A tremendous amount of knowledge has been accumulated about this organism, which today can be claimed to be the best understood of all eukaryotes, if not the best understood organism of all (at least from a molecular biology point of view). This wealth of knowledge is now available to apply to further the development of wine yeast strains (Bauer and Pretorius 2002).

Technically, there are two fundamental differences between genetic engineering approaches and all other previously described methodologies.

The first is the high specificity of the genetic engineering approach. Hybridization, directed evolution and mutagenesis are random methods, requiring long selection procedures, and when desired strains are obtained, no information regarding the biological and molecular changes that have led to the emergence of the desired trait is available. In the case of genetic engineering, the contrary is the case, as significant information regarding the molecular foundation (i.e., the genes, their positions and their regulation) of the trait to be modified or to be improved must exist beforehand.

The second fundamental difference from the previous methods is the ability to impart new, completely different

characteristics to a wine yeast strain; characteristics that may be absent from *S. cerevisiae* or any closely related species.

Potential for future application

What is the potential of the different technologies described above to contribute to the improvement of commercial wine yeast strains? Currently, the market requires a diversity of yeast specifically adapted to local conditions and satisfying the need to produce specific types and styles of wine. But beyond these immediate concerns of the current market, there is also a need to prepare for possible future development and new consumer demands. To satisfy such future demands, methods that can impart completely new and different characteristics regarding the processing of wine (enzymes), the preservation (alternatives to SO₂), or the flavour, aroma and health aspects of wine (nutraceuticals), may be required.

Two general considerations regarding the potential of yeast strain development strategies to fulfill those demands can be made.

The first consideration relates to the complexity (the total amount of different nucleotide sequences, i.e., alleles and genes, that is available and can be combined when using a specific technology) of the available DNA. Indeed, our ability to generate new strains and new traits in any given species is dependent on the availability of DNA which carries the specific traits. As a consequence, any given strain development technique is limited by the degree of complexity of the available source DNA. In the case of isolates, this means that any selection program is limited to those combinations of DNA that occur naturally. In the case of hybrids, our ability to source and bring together DNA from strains isolated from different areas (and therefore evolutionarily more distant), and from different, although always closely related species, increases the complexity of the source DNA and therefore allows the development of a larger variety of strains than an approach limited to the isolation of natural yeast. However, it is clear that from this point of view, genetic engineering offers by far the largest variability. Indeed, source DNA is virtually unlimited, since DNA present in any genome of any organism can theoretically be used to further develop specific traits of wine yeast strains.

A second general consideration relates to the genetic nature of the traits to be modified or improved. Many of the important characteristics of wine yeast are so-called polygenic traits, meaning that a specific phenotype is due to the combined action and specific interaction of

many, sometimes hundreds of genes. However, and as Table 1 indicates, many other important characteristics are dependent on relatively few, sometimes even on single genes. The polygenic or otherwise nature of a trait will have tremendous influence on which technology has the most promising outlook to deliver a desired result. Since polygenic traits are difficult to analyze on a molecular level, genetic engineering is frequently not able to easily modify such traits. It is indeed difficult or impossible to identify the relevant target genes that have to be modified to achieve a specific outcome.

Some random approaches, and in particular various hybridization strategies, are therefore certainly more likely to succeed in improving such traits. This is particularly true if a strong selective pressure can be applied to enrich and select the improved strains. However, with new molecular methodologies becoming available, in particular the techniques of global analysis also referred to as functional genomics, our understanding of complex traits is improving rapidly, suggesting that the identification of specific target genes to be modify such traits will soon be possible.

Beyond these general points, there are specific factors that define the potential future usefulness of various strategies.

The isolation of strains and the selection of naturally occurring variants will certainly continue to play a role in future developments. The genetic variability of the *Saccharomyces* species, with an established high degree of allelic heterozygosity, clearly allows the generation of many, highly variable strains fulfilling many very specific criteria. Most commercial wine yeast strains currently sold are isolates. They are fulfilling all the basic requirements that winemakers expect of yeast. However, the desire of wine yeast producers to offer better, optimized strains, and strains better adapted for specific purposes, suggests that such strains do not always offer the right combination of characters to satisfy the increasing expectations. While further isolation programs may identify new strains with different and new properties, other strategies based on scientifically designed breeding and selection programs or genetic engineering appear indispensable.

In the past decade, hybrid yeast strains have been highly successful, indicating that the specific characteristics obtained through a scientific approach to breeding can produce outcomes that satisfy the needs and requirements of winemakers. Some hybrids display superior abilities for at least some important traits when compared to the available isolates. Future breeding and selection programs will certainly lead to the development of new strains with

improved characteristics for many of the traits listed in Table 1. However, it must be kept in mind that the possibility of achieving specific outcomes, while significantly increased when compared to the simple use of isolates, remains limited by the genetic potential of *S. cerevisiae* and other closely associated species. Indeed, it is impossible to introduce fundamentally new traits through the use of these technologies.

A similar argument applies to the use of mutagenesis. The technology certainly has the potential to improve existing strains for specific characteristics, but again the technique does not allow for the introduction of new or different traits than those already present in the genome of the parental strain (although, and as for hybridization and as a matter of principle, the emergence of new traits is an evolutionary possibility).

Only GM has the potential to introduce specific new functions or traits into a given wine yeast strain. A look at Table 1 highlights that many of the suggested improvements of yeast strains, particularly those related to the nutritional value of the product and the preservation thereof, can be achieved only through the use of this approach. The particular power of the GM approach lies in the availability of a virtually unlimited amount of source DNA, representing all imaginable traits or characteristics. This opens new possibilities beyond the traits highlighted in this review. A further potential advantage of GM is that the modification can be introduced into existing wine yeast strains that in theory are not further modified through the manipulation, and should keep the specific traits that made the “parental” yeast a good wine yeast in the first place. However, recent data show that this is not always the case as most modifications involve a redirection of metabolic flux leading to potentially significant changes in traits unrelated to the one being added or modified. Nevertheless, it is clear that the chief limitations of GM approaches are mainly of a non-scientific nature, including legal and regulatory issues as well as aspects of wine marketing.

Conclusion

This review is concerned mainly with the scientific and technological potential of various yeast improvement methodologies. However, it is clear that many other factors influence the choice of a strategy when developing a new strain. Essential factors which were not discussed here include the regulatory or legal framework of individual wine-producing countries, trade regulations, and perceptions in the market place. For most of these factors, there is a clear dividing line between genetic engineering and all other methodologies. Only genetically modified

yeast has to undergo a lengthy approval process, including a detailed evaluation of the impact on the product (chemical composition, health aspects) and on the environment. Even if approved by individual countries, genetically modified yeast has not received International Organisation of Vine and Wine (OIV) approval at this stage. Yeast developed through any other methodology does not require any evaluation or approval and can be sold without further evaluation. Whether such a framework makes scientific sense is doubtful. Indeed, the potential inherent risks of random technologies may well be higher than perceived risks associated with well controlled genetic engineering approaches.

The wine market is certainly not ready to accept genetically modified yeast strains at this stage, although a significant percentage of winemakers and drinkers would probably be curious enough to give it a try. Further studies are currently underway to evaluate these perceived risks and should provide a sound scientific base for future debate (Bauer et al. 2004). Many yeast strain development programs based on traditional breeding and selection methodologies are therefore underway, and new strategies are emerging that should lead to more focused outcomes.

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TABLE 1. Improvement of wine yeast strains: Target categories

Properties	Methods that can be applied (preferred method if relevant)	Comments
Fermentation performance		
General resilience and stress tolerance	All (hybridization)	Polygenic trait. Strong selection pressure can be applied, and non-GM breeding programs offer good potential for further improvements.
Efficiency of sugar utilization	All (mutagenesis)	Polygenic trait. Strong selection pressure can be applied, and non-GM breeding programs offer good potential for further improvements.
Efficiency of nitrogen utilization	All (hybridization, genetic engineering)	Polygenic trait. Strong selection pressure can be applied, and non-GM breeding programs offer good potential for further improvements. However, if the use of a specific N-source should be improved (proline), GE can provide a safer, faster option since only a limited number of genes are required.
Conduct malolactic fermentation	Genetic engineering	Only option. First GM yeast with FDA approval.
Improved processing efficiency		
Must yield, clarification and extraction of colour and aroma compounds	Genetic engineering	Expression of specific enzymes (proteases, glucanases, pectinases etc). <i>S. cerevisiae</i> produces only a limited number of these enzymes, providing limited leverage for hybridization or mutagenesis approaches. The most efficient method is clearly through GE. A significant number of GE strains have been generated and proven their efficiency in small-scale wine production.
Controlled cell sedimentation and flocculation	Genetic engineering	Controlled expression of flocculation genes.
Biological control of wine spoilage microorganisms		
Wine yeasts producing antimicrobial enzymes or peptides	Genetic engineering	No other method, as genes encoding such proteins/peptides are not present in <i>S. cerevisiae</i> .
Wine wholesomeness		
Increased production of antioxidants or other nutraceuticals	Genetic engineering	No other method, as metabolic pathways are not present in <i>S. cerevisiae</i> .
Reduced formation of ethyl carbamate	Mutagenesis	Deletion of single gene can be achieved through non-GM approaches.
Decreased yield of ethanol	All traditional methods/ Genetic engineering	Several strategies have been implemented with variable success. No non-GM yeast with significantly reduced ethanol yields has been generated thus far.
Wine sensory qualities		
Enhanced liberation of grape terpenoids	All traditional methods/ genetic engineering	GE will be the more successful methodology, but some success can be achieved through traditional methods.
Optimized production of aroma, flavour and mouthfeel compounds	All traditional methods/ genetic engineering (metabolic engineering)	Large number of metabolites, including volatile aroma compounds (esters and higher alcohols) and flavour compounds (acid balance, etc). To achieve a specific outcome, GE is clearly the most promising option. However, every yeast strain produces a specific aroma profile, and traditional methods will allow generating a wide range of aroma production capacities.

YEAST'S CONTRIBUTION TO THE SENSORY PROFILE OF WINE

TABLE 2. Methods for strain development

Method	Brief description of the method
Isolation and selection of variants	Direct method, based on selection of yeast strains present in spontaneous fermentation. Genetic variants found within populations of all wine yeast strains due to spontaneous mutations can be selected if selection pressure can be applied.
Hybridization	<p>Intra-species hybridization entails sporulating diploids, recovering individual haploid ascospores and mating of haploid cells of opposite mating types to produce a new heterozygous diploid. As for all sexual reproduction, the resulting diploid strains may show properties that are different from that of either parental strain. Hybridization is effective for improving and combining traits under polygenic control. The inclusion or elimination of a specific property can be achieved fairly quickly by hybridization, on the condition that the property has a simple genetic basis, for example, one or two genes.</p> <p>There are several forms of specialized types of hybridization in cases where classical sexual reproduction cannot be applied. These include:</p> <p>Spore-cell mating: Many wine yeast strains are homothallic and require a direct spore-cell mating procedure, which entails placing four homothallic ascospores from the same ascus in direct contact with heterothallic haploid cells by micromanipulation. Mating will occur between compatible ascospores and cells.</p> <p>Rare mating: Forcing mating of strains that do not express a mating type.</p> <p>Spheroplast fusion: Direct, asexual technique. The procedure overcomes the requirement for opposite mating types. It involves the enzymatic removal of the cell wall, and mixing the spheroplasts from different parental strains in the presence of a fusion agent. Two diploid wine yeasts with complementary desirable traits can be fused to generate a tetraploid wine yeast strain that includes the entire genetic backgrounds of the two parental strains.</p>
Mutagenesis	Application of mutagens to increase the frequency of mutations in a wine yeast population. High frequency of mutations can lead to multiple phenotypes, certain traits being improved, while others are simultaneously debilitated.
Genetic modification (GM) or engineering (GE)	The use of recombinant DNA technology and genetic engineering to change specific properties of a wine yeast strain. Transformation offers the possibility of precisely changing specific characteristics. An existing property can be modified, a new characteristic can be introduced, or an unwanted trait can be eliminated. Detailed knowledge is required about the genetic background of the host strain, the cellular mechanisms and structures that contribute directly or indirectly to the expression of the modified or heterologous genes, and the activity and metabolic role of the protein encoded by the gene(s).

ROLE OF YEAST IN THE HYDROLYSIS OF GLYCOSIDICALLY BOUND VOLATILE COMPOUNDS DURING WINEMAKING

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Introduction

The aroma characteristics of wine are strongly dependent on the pool of odour-active compounds that migrate from grapes to wine during winemaking. Many of these volatile constituents, including such potent wine odorants as terpene alcohols, C-13 norisoprenoids and shikimic acid derivatives, are present in grapes mainly as non-volatile, flavourless, sugar-bound conjugates (glycoconjugates) and, to a lesser extent, as free volatile constituents. The terpene alcohols, for example, make an important contribution to the varietal characteristics of the “floral” grape varieties (Muscats, Riesling and Traminer) (Strauss et al. 1986). In the case of the so-called “neutral” or “non-floral” grape varieties, the concentrations of free, odour-active forms of these compounds are usually very low, and in addition, they are largely present as odourless glycosidic precursors (Sefton et al. 1993). For this reason, juices obtained from these grape varieties generally lack a distinctive or typical odour. Nevertheless, the resulting wines very often exhibit aroma characteristics that are specific to the grape variety employed for winemaking, suggesting that the vinification process can reveal varietal sensory characters of the fruit. Several mechanisms have been proposed with the most important being the transformation of odourless precursors into fragrant compounds. Acid hydrolysis, by the acids present in wine (Williams et al. 1982, Günata et al. 1988), enzymatic hydrolysis, by added enzymes or those derived from the grape or microorganisms (Williams et al. 1982, Günata et al. 1985, 1988), and biosynthesis of aroma compounds by yeast (Carrau et al. 2005) can be involved.

Glycosidic precursors of grapes include monosaccharide glycosides, in which the sugar moiety consists of a β -D-glucose unit, and disaccharides, in which the glucose is further substituted with a second sugar unit, typically α -L-arabinofuranoside, β -D-rhamnopyranoside, or β -D-apiofuranoside (Günata et al. 1988). Under the mild acidic conditions of wine, spontaneous hydrolysis of the β -glucosidic linkage of glycosides results into the release of the bound volatile compounds (Sefton et al. 1993). Although this process is known to have a primary role in the development of wine aging bouquet, it is generally slow, and is therefore thought to have a minor impact on the development of varietal character of non-aged wines. On the contrary, it is known that several microorganisms can produce glycosidase enzymes able to promote the rapid hydrolysis of grape glycosides and the consequent release of the bound odour-active fraction. It is generally accepted that complete enzymatic hydrolysis of disaccharide glycosides requires the preliminary action of an appropriate glycosidase (arabinosidase, rhamnosidase, xylosidase or apiosidase) to release the terminal sugar, before the β -glucosidase is able to release the bound volatile fraction. Only this latter step is needed for glucosidic precursors (Günata et al. 1988). Several *in vitro* studies conducted with *Saccharomyces cerevisiae* have shown that strains of this yeast possess the glycosidase enzymes needed to liberate the volatile fraction of glycosidically bound precursors of grape, although these enzymes are unstable or have low activity at wine pH, and may be inhibited by glucose and high concentrations of ethanol (Delcroix et al. 1994, Rosi et al. 1994, Charoenchai et al. 1997). Nevertheless, other authors have suggested that, at least in the

early stages of alcoholic fermentation, the β -glucosidase of *S. cerevisiae* could actively contribute to the liberation of grape-derived volatile compounds from glycosides (Darriet et al. 1988, Mateo and Di Stefano 1997).

A series of experiments were undertaken to better define the contribution of *Saccharomyces* yeast to the hydrolysis of grape glycosides and the consequent release of volatile compounds during winemaking. Experimental design involved the use of a chemically defined grape juice (CDGJ) medium containing glycosides directly extracted from grape juice, in order to simulate winemaking conditions typically found during the production of wines from non-floral grape varieties. For some of the yeast strains tested, experiments were also carried out with whole grape juice. Part of the results of these studies is reported in the following sections.

Evaluation and characterization of the hydrolytic activity of *Saccharomyces* yeasts during fermentation

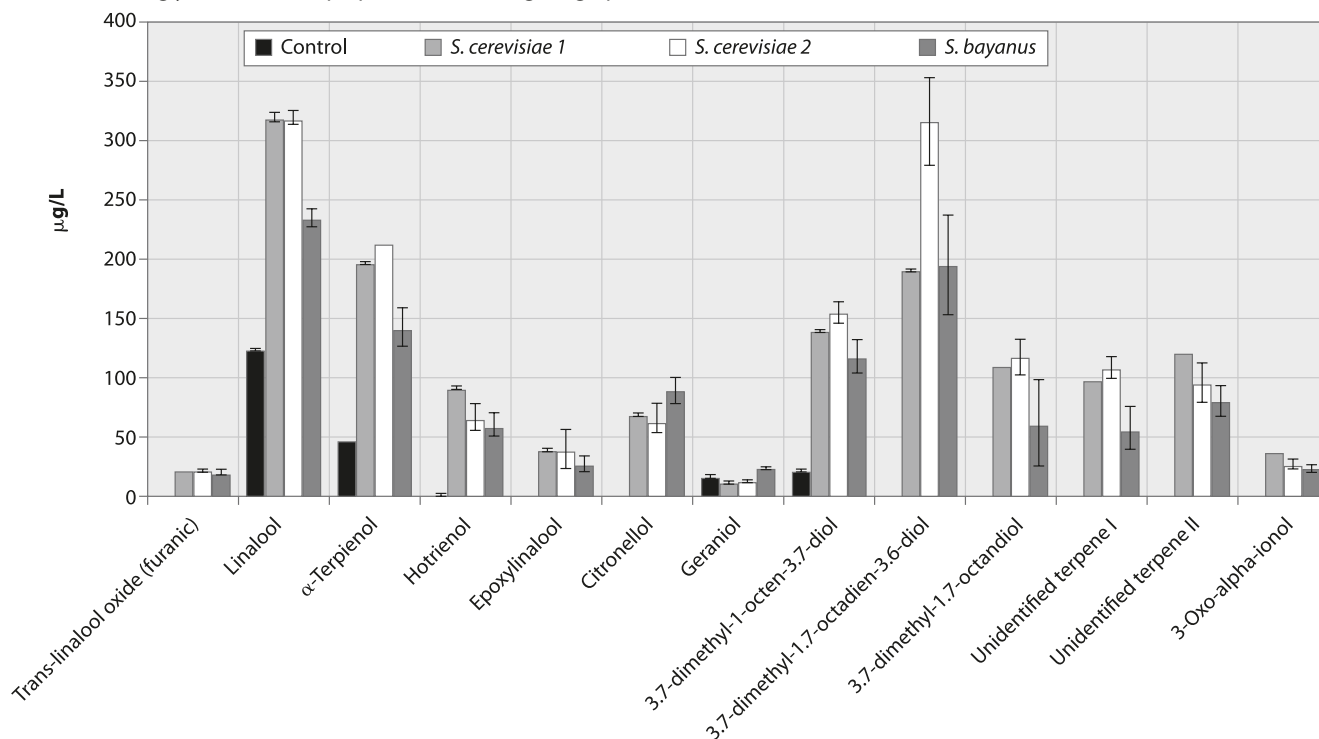
The first experiment involved the use of a grape-derived glycosidic extract added to a chemically defined medium so that yeast-derived glycosidic enzymes could be studied under defined conditions and in the absence of exogenous enzymes that might be present from other sources. The chemically defined grape juice (CDGJ) medium used was similar to that described by Henschke and Jiranek (1993),

but without lipids; glucose plus fructose was 200 g/L, and the pH was 3.2. Glycosides were extracted from a freshly prepared *Vitis vinifera* Frontignac (Muscat) juice obtained from a winery according to the method of Williams et al. (1982, 1992). Two *S. cerevisiae* and one *S. bayanus* strains were investigated, and were obtained from the Australian Wine Research Institute culture collection. Fermentations were conducted in filter sterilized media incubated at 18°C in 250 mL Erlenmeyer flasks closed with fermentation locks and shaken at 180 oscillations per minute. Samples were removed with a needle and syringe via a sample port closed with a rubber Suba seal.

For the analysis of volatile compounds, samples were spiked with 2-octanol as the internal standard, and extracted with dichloromethane. The organic extracts were analyzed by GC-MS under the conditions reported by Ugliano et al. (2003).

The concentrations of some Frontignac aglycones, as determined by GC-MS analysis, of wines produced by fermentation with three different yeast strains, are shown in Fig. 1. Fermentation resulted in a significant increase in the concentration of several volatile compounds compared to the uninoculated control. The increase of volatiles due to non-enzymic (spontaneous or acid-catalyzed) hydrolysis of glycosides (Control) was, as expected, lower than that associated with alcoholic fermentation. Moreover, none of the compounds in Fig. 1 were detected in CDGJ medium

FIGURE 1. Effect of alcoholic fermentation and *Saccharomyces* yeast on the volatile fraction of model wines. Model media were supplemented with a glycosidic extract prepared from Frontignac grapes.



samples that did not receive glycosides (data not shown). These results indicate that, while chemical hydrolysis of glycosides plays a minor role in the hydrolysis of glycosidically bound volatile compounds during vinification, yeast can actively contribute to the process of transformation of non-volatile precursor forms into volatile compounds. An association between yeast strain and differences in the concentrations of some volatiles was found (Fig. 1), although too few strains were studied to make a generalization. The compounds detected in the wines, such as linalool, α -terpineol, and citronellol, were of great interest, as at the end of fermentation these compounds occurred at concentrations near to or exceeding their odour thresholds. The detection of citronellol is also interesting, as this compound does not derive directly from glycosides, but is in fact produced by yeast through the transformation of geraniol (Di Stefano et al. 1992). Its occurrence in the fermented samples indicates therefore that, following the cleavage of the glycosidic linkage, yeasts can promote further transformations of the aglycon moiety of glycosides. Citronellol can also, at least in part, be derived by *de novo* synthesis from yeast lipid metabolism (Carrau et al. 2005), although under the experimental conditions of this study we did not observe production of citronellol in ferments without glycosides. As for other volatiles, the release of terpene diols might play a role in the expression of grape varietal aromas. Although the direct contribution of these compounds to the aroma of wine is negligible, under the mild acidic conditions of wine they act as precursors to other wine odorants, such as monoterpene alcohols (Williams et al. 1980).

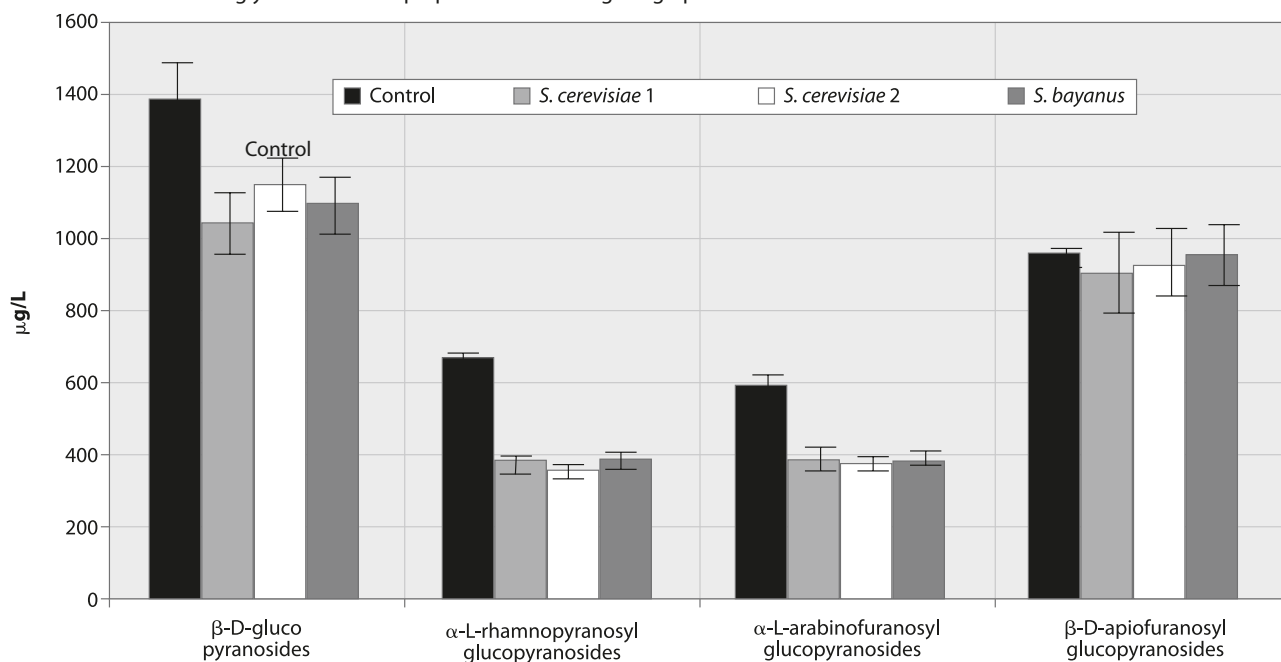
The specificity of yeast hydrolytic activity towards the different glycosides present in grapes and wines was also investigated. This was achieved by direct analysis of trifluoroacetylated derivatives of the residual glycosides present in wines after alcoholic fermentation (Voirin et al. 1992). The sugar moiety was shown to be a key factor in the extent of hydrolysis of different glycoside classes (Fig. 2). The decrease of β -D-glucopyranosides, α -L-arabinofuranosides, and β -D-rhamnopyranosides was generally higher than for β -D-apiofuranosides. No significant differences between the three strains tested were observed. These observations indicate that production and/or activity of enzymes specific for apioside substrates might be limited in *Saccharomyces* yeast, at least in the three strains tested, during fermentation.

The influence of yeast strain on free and glycosidically bound volatile compounds of wine

In another study, the behaviour of the free and glycosidically bound forms of four grape-derived monoterpene alcohols with high sensory impact (linalool, geraniol, nerol, and α -terpineol) was investigated during fermentation with four commercial *S. cerevisiae* starter cultures. Several other terpenes were also measured during the same experiment. Fermentations were carried out using the CDGJM described in Table 1, and a glycosidic extract obtained from Muscat grape juice.

At the end of alcoholic fermentation, volatile compounds of wines were extracted as described on page 48. Confirm-

FIGURE 2. Effect of alcoholic fermentation and *Saccharomyces* yeast on the glycosidic fraction of model wines. Model media were supplemented with a glycosidic extract prepared from Frontignac grapes.



ing the results of the previous experiment, a significant increase of volatile compounds resulting from the hydrolysis of glycosides was observed, as shown in Fig. 3, for the most abundant classes of terpene compounds. Moreover, in this case, the final concentration of different volatiles

TABLE 1. Composition of the CDGJ medium

Compound	Amount
Sugars (g/L)	
Glucose	100
Fructose	100
Acids (g/L)	
Tartaric	3
L-Malic	2
Nitrogen compounds and minerals (mg/L)	
KH ₂ PO ₄	500
CaCl ₂ · 7H ₂ O	100
NaCl	100
(NH ₄) ₂ SO ₄	500
(NH ₄) 2HPO ₄	500
Vitamins (mg/L)	
Biotin	0.5
Calcium pantothenate	1
Thiamin	0.3
Nicotinic acid	3
pH	3.2 (NaOH)
Glycosidic extract obtained from Muscat grape juice was added where required	

was dependent on both the yeast strain and the chemical structure of the compounds. Wines obtained with strains 1 and 4 were characterized by higher concentrations of terpenes at a higher oxidation state (sum of linalool oxides and epoxydes), while tertiary terpene alcohols (sum of linalool and α-terpineol) reached a higher concentration with strain 3. Wines obtained with strain 2 were generally characterized by low concentrations of all the different terpene classes measured, except for terpene diols (sum of 3.7-dimethyl-1.5-octadien-3.7-diol, 3.7-dimethyl-1.7-octadien-3.6-diol and *trans*-8-hydroxylinalool).

Glycosides remaining in the experimental samples at the end of fermentation were extracted by means of C18 SPE cartridges and recovered with methanol. Solvent was removed by means of a rotary evaporator, samples were re-dissolved in phosphate-citrate buffer at pH 5.0 and treated with a commercial preparation of glycosidase enzymes (AR 2000, Gist Brocades). The volatile compounds in these enzyme hydrolisates were extracted and analyzed by GC-MS, as described above.

Surprisingly, the concentration of glycosides of individual volatile compounds at the end of fermentation did not match the profile obtained for free volatile compounds. Fig. 4 shows this trend for the four main terpene alcohols. Considering that strain 2 was generally characterized by a lower release of volatiles, wines obtained with this yeast were expected to have higher concentrations of residual bound precursor forms. On the contrary, the concentration of glycosides at the end of fermentation in wines obtained with strain 2 were similar, if not lower, than those observed in wines obtained with the other strains. These results indicate that the hydrolytic activity of yeast is not the only factor affecting the composition of both free and

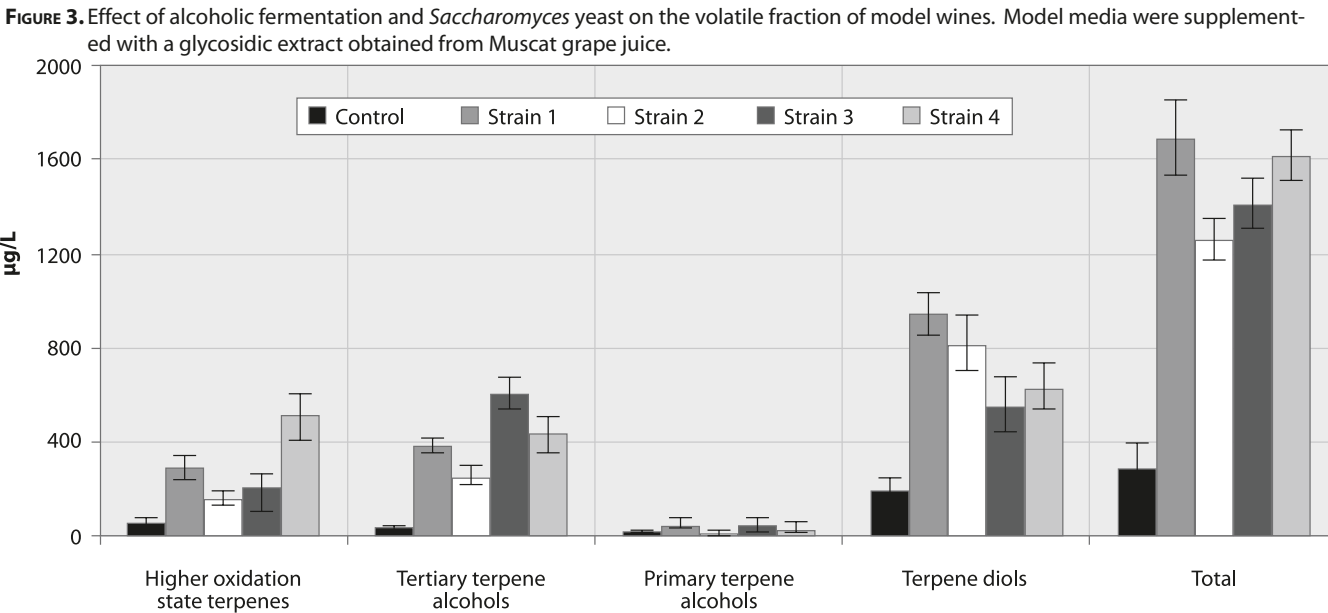
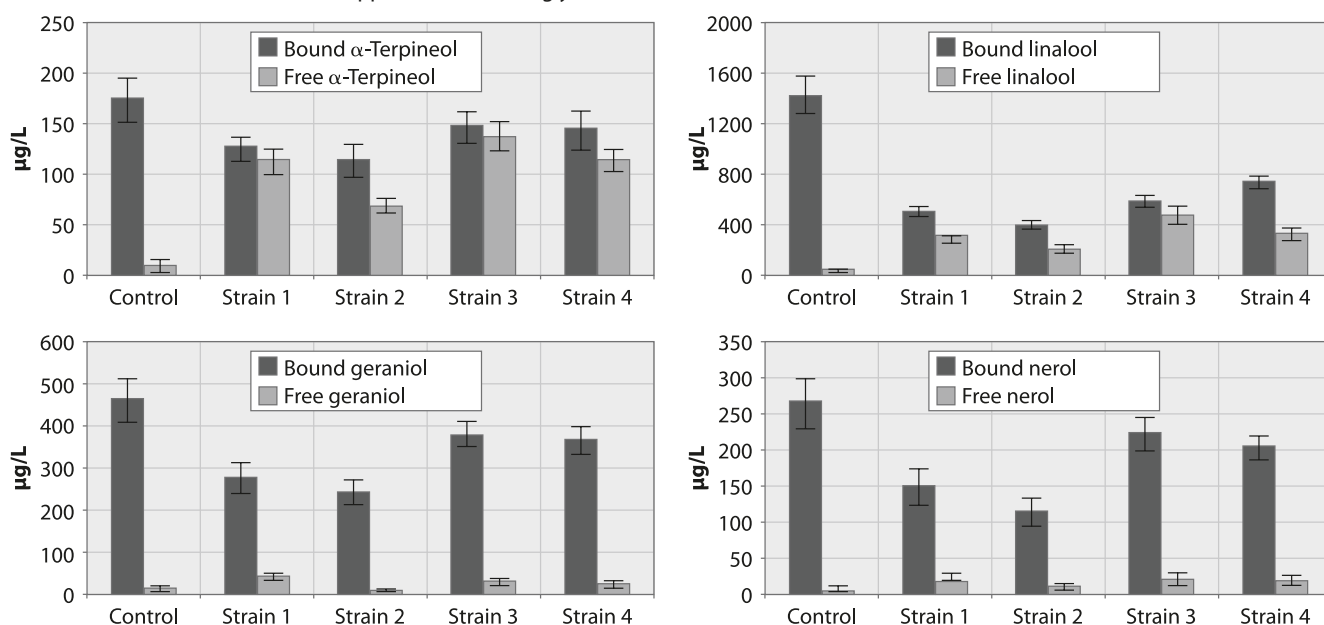


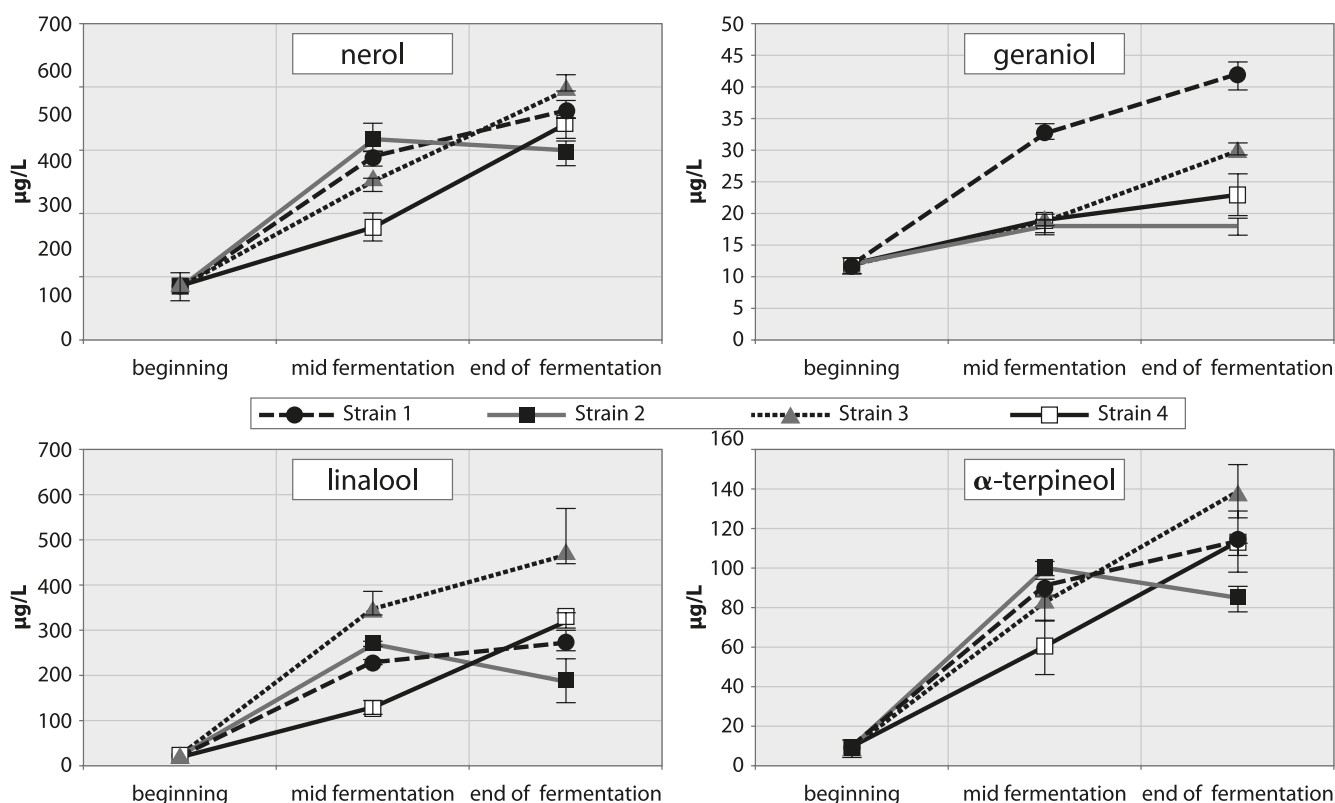
FIGURE 4. Effect of *Saccharomyces* yeast strain on the concentration of free and glycosidic forms of the four main terpene alcohols model wines. Model media were supplemented with a glycosidic extract.



bound varietal volatile fractions of wine. The evolution of the four main grape-derived terpene alcohols during fermentation (Fig. 5) suggests the existence of other factors accounting for the different levels of aglycons observed in the synthetic wines. The lower levels of terpene alcohols found in wines obtained with strain 2 at the end of fermentation were in fact due to a decline in the concentra-

tion of these compounds occurring in the second part of fermentation. Other interactions such as metabolization of the liberated aglycons by yeast cells and adsorption onto yeast walls are therefore likely to play an important role in the process of transformation of glycosidic precursors into volatile compounds. For example, the decline of terpenols concentration observed for strain 2 might be linked to ad-

FIGURE 5. Evolution of the free forms of the four main terpene alcohols.



sorption phenomena of these volatiles on yeast derived macromolecules, whose release into the growth medium is known to occur during the decline phase of yeast cells (Guilloux-Benatier et al. 1995).

Yeast strains 1 and 2 were also tested during vinification of the neutral grape variety Falanghina. Consistent with the behaviour observed during the study with the CDGJ medium, wines obtained with strain 2 exhibited lower concentrations of glycosides after alcoholic fermentation, although the concentrations of free terpene alcohols were similar, if not lower in some cases, than those observed for the wines obtained with strain 1 (Table 2).

TABLE 2. Concentration of free and bound volatile compounds and total glycosides in Falanghina wines at the end of fermentation with commercial yeast strains 1 and 2

	Strain 1	Strain 2	Sig. ^a
Free volatiles (µg/L)			
Linalool	19	19	ns
α-terpineol	8	3	*
Geraniol	8	5	*
Total	34	27	*
Bound volatiles (µg/L)			
Linalool	24	19	*
α-terpineol	5	5	ns
Nerol	12	11	ns
Geraniol	85	83	ns
Benzyl alcohol	35	35	ns
2-phenylethanol	103	88	*
Eugenol	22	8	*
Total	285	249	*
Total Glycosyl-glucose(µM)			
	52	39	*

^aSignificance: ns=not significant; *=significant at $p<0.05$

Conclusion

These studies provide clear evidence for the role played by *Saccharomyces* yeasts in the transformation of odourless (non-volatile) glycosidic precursors of grapes into odour-active volatile compounds that can potentially contribute to the aroma characteristic of wine. This work also highlights the need for further research in this field in order to optimize the criteria for the selection of yeast that give more effective expression of the varietal aroma character of wine. Both *S. cerevisiae* and *S. bayanus* yeasts posses the enzyme activities necessary to hydrolyze glycosidically bound volatile compounds during alcoholic fermentation. The fact that the extension of this hydrolytic activity is dependent on the chemical structure of the sub-

strate can have important technological implications, due to the well-known influence of the grape variety on the chemical composition of the pool of glycosidically bound volatile compounds of grapes (Voirin et al. 1992). Specifically, the relative stability of apiosylglucosides during alcoholic fermentation might result in lower hydrolysis of glycosidically bound volatiles for grapes containing high proportions of this type of substrate.

The ability of yeast to hydrolyze glycosides of volatile compounds was also strain dependent, which indicates the need for further studies aimed to the recognition of strains with high glycosidase activity. Moreover, yeast growth and metabolism can promote other processes affecting both the size and the composition of the pool of volatile compounds released into wine as a result of glycoside hydrolysis. These processes can include enzymatic transformation and/or complete metabolization of the liberated aglycon, and adsorption on macromolecules released during fermentation. The possibility that these processes can also take place with dynamics that are strain-dependent imposes, in the future, experimental approaches aimed not only to evaluate the intrinsic ability of different yeast strains to release volatile compounds from glycosidic precursors, but envisaging all the different aspects of yeast growth and metabolism during winemaking. In this sense, it must be borne in mind that a low degree of enzymatic hydrolysis of glycosides during alcoholic fermentation should not necessarily be considered as detrimental for the expression of wine varietal character. Glycosides remaining after fermentation will undergo slow spontaneous hydrolysis during wine aging, a process that imparts aroma complexity to wine, as it results in the formation of a pool of volatile compounds with sensory characteristics different from the ones obtained through enzymatic hydrolysis (Sefton et al. 1993). Optimal exploitation of the contribution of yeast to the expression of the grape varietal character therefore involves knowledge of the characteristics of the grape variety in question and the careful choice of the style of wine expected.

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SELECTED YEAST UTILIZATION AND BIODIVERSITY

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Abstract

The use of commercial wine yeast strains as starters has become widespread over the past two decades. These wine yeast strains are released in wineries on an annual basis. However, little is known about the fate of these strains in the vineyard. To evaluate the ability of industrially produced starter yeast to survive and spread in nature, and to naturalize – becoming part of the natural microflora of musts – we devised a large-scale sampling plan over a period of three years in six different vineyards (three in Portugal and three in France). Each vineyard had used the same industrial yeast strain(s) continuously over the last five years. A total of 198 grape samples were collected at various distances from the wineries, before and after harvest. Towards the end of the spontaneous fermentations, the composition of the yeast flora was determined by different typing methods (PCR-amplification of * *-sequences, pulse field electrophoresis, RFLP of mitochondrial DNA, and microsatellite typing). Among the 3,780 yeast strains identified, 296 isolates had a genetic profile identical to that of commercial yeast strains. For a large majority (94%), these strains were recovered at very close proximity to the winery (10-200 m). Commercial strains were mostly found in the post-harvest samples, reflecting immediate dissemination. Analysis of population variations from year to year indicated that permanent implantation of commercial strains in the vineyard did not occur, but instead that these strains were subject to natural fluctuations of periodical appearance/disappearance like autochthonous strains. Overall, the data show that dissemination of commercial yeast in the vineyard is restricted to short

distances and limited periods of times and is largely favoured by the presence of water runoff.

Introduction

Since the beginning of the 1980s, the use of active dried *Saccharomyces cerevisiae* yeast starters has become generalized. Today, the majority of wine production is based on the use of active dry yeast, which ensures rapid and reliable fermentation, and reduces the risk of sluggish or stuck fermentation and of microbial contamination. Most commercial wine yeast has been selected in the vineyard for such oenological traits as fermentation performance, ethanol tolerance, the absence of off-flavours and production of desirable metabolites. These and other technological developments have contributed to improving wine quality, and have enhanced the ability of winemakers to control the fermentation process and achieve specific outcomes.

Commercial yeasts are classically used in winemaking without any special containment and are released annually in large quantities, together with liquid and solid winemaking residues, in the environment around the winery. The behaviour of these yeasts in the ecosystem of the vineyard is totally unknown as is their potential impact on the natural microflora. In particular, it is not known if commercial strains are able to survive in nature and join the vineyard microflora. Only very few data are available that could contribute to the evaluation of the importance of starter yeast dissemination and permanence in the vineyard (Frezier and Dubourdieu 1992; Vezinhet et al. 1992; Guilla  n et al. 1996). Recently, a large-scale bio-

geographical study in South African vineyards was carried out in five areas situated in the Coastal Region vineyards of the Western Cape. Commercial yeasts were recovered in three of 13 samples (van der Westhuizen et al. 2000a and 2000b).

The present large-scale study, carried out in different geographical sites of France and Portugal, aims to evaluate the ability of industrially produced starter yeast to spread and survive in nature. The data will serve as a strong basis to evaluate whether inoculated strains join the natural microflora and affect biodiversity, and whether they influence fermentation in the following years, especially fermentation performed according to traditional practices that rely on spontaneous fermentation. Such data will also serve as strong basis to evaluate potential risks associated with the use of genetically modified (GM) yeasts.

Methodology

The sampling plan included 36 sites in six vineyards, three located in the south of France (Languedoc) and three in the north of Portugal (Região Demarcada dos Vinhos Verdes). The overall duration of these studies is three years (2001-2003). The wineries selected used consecutively one or more commercial yeast strains in the past five years. The three Portuguese wineries used mainly Zymaflore VL1, a strain originally selected in France, while the three French wineries used predominantly K1M ICV-INRA. A total of 34 commercial wine yeast strains were used in the six wineries during the three-year study.

In each vineyard, six sampling points were defined according to local conditions (size and orientation of the vineyard, predominating wind direction). The distance between winery and the sampling sites varied from 20 to 1,000 m. In order to evaluate the remanence over years of commercial yeast, a first sampling campaign was performed before the winery started wine production with the use of commercial yeast strains (pre-harvest samples). In a second post-harvest sampling campaign, the grapes were collected, after the onset of wine production, in order to evaluate the immediate commercial yeast dissemination from the winery. With the present experimental design, 72

grape samples were collected each year. From each sampling point, approximately 2 kg of grapes were aseptically collected, and the extracted grape juice was fermented in small volumes (200-500 mL), with mechanical agitation at 20°C. Daily weight determinations allowed the monitoring of the fermentation progress. The yeast flora was analyzed when the must weight was reduced by 70 g/L, corresponding to the consumption of about two thirds of the sugar content. Must samples were diluted and spread on plates with YPD medium (yeast extract, 1% w/v, peptone, 1% w/v, glucose 2% w/v), and after two days of incubation 30 randomly selected colonies were collected from each spontaneous fermentation. The *Saccharomyces* strains were first selected on a selective medium with L-lysine as sole nitrogen source. The *Saccharomyces* not able to grow on L-lysine medium were subjected to molecular identification based on mitochondrial DNA restriction profiles (Querol et al. 1992), microsatellite analysis using six loci (ScAAT1-ScAAT6) (Perez et al. 2001), karyotype pattern using pulsed field gel electrophoresis (PFGE) (Blondin and Vezinhet 1988) and interdelta sequence amplification patterns (Ness et al. 1993, Legras and Karst 2003). Before starting the study, we evaluated the discriminatory power of different typing methods on a total of 23 commercial yeast strains used in the wineries of the two countries. Among the 23 commercial yeast strains analyzed, 22 different patterns were obtained using karyotyping analysis and 21 using the three other methods (Schuller et al. 2004). Due to the verified similarity of the discriminatory power of these methods, any of them could be used for our study and the results obtained will be comparable.

Results

A total of 198 samples were collected during three consecutive campaigns (2001-2003), 108 of which were taken in France and 90 in Portugal (Table 1).

Of the 198 samples, 126 musts (64%) produced spontaneous fermentations, 20% and 44% in must from pre-harvest and post-harvest campaigns respectively. The percentages of spontaneous fermentations were similar in both countries, 66% in France and 60% in Portugal. A total of 3,780

TABLE 1. Distribution of global data by country and year

	2001		2002		2003		Total
	France	Portugal	France	Portugal	France	Portugal	
Samples	36	36	36	18	36	36	198
Spontaneous fermentations	24	19	33	12	15	23	126
Isolates	720	570	990	360	450	690	3780
<i>Saccharomyces</i> strains	406	570	120	360	209	690	2355

TABLE 2: Commercial yeast strains recovered in each vineyard over the three years

Vineyards	A	B	C	D	E	F	Total
Spontaneous fermentations	19	24	29	16	23	15	126
Spontaneous fermentations with ≥ 1 commercial yeast strains	0	2	1	11	9	2	25
Isolates	570	720	870	480	690	450	3780
Commercial yeasts strains	0	15*	1	206	54+18*	2	296
% Commercial yeast / nb of isolates	0	2	0.1	43	10	0.5	7.8

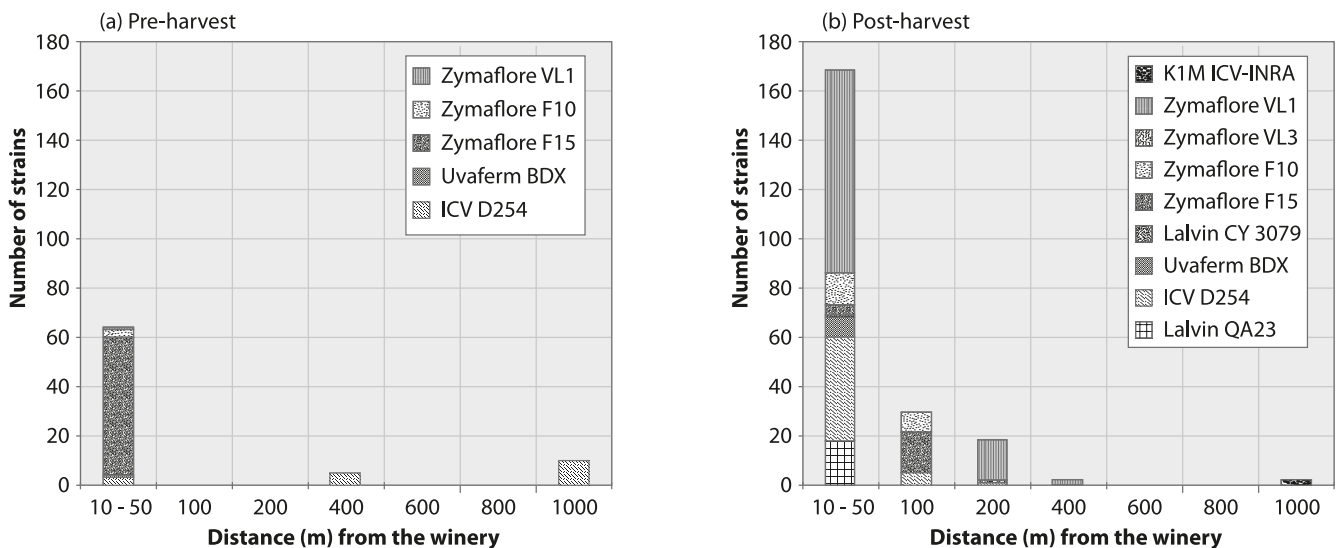
* Strains originated from the same area

colonies were isolated, of which 2,355 were identified as *Saccharomyces* strains.

Molecular characterization of the 2,355 *Saccharomyces* isolates led to the identification of 296 strains with a genetic profile similar to that of commercial yeasts (Table 2). These strains represent 7.8% of the fermentative yeast community, the majority of which (5.8%) were recovered in post-harvest campaigns. It should be noted that since fermentation is used as an enrichment tool for *Saccharomyces* strains, the present results do not allow conclusions about the number of strains occurring on the surface of the grape, which is in fact very low. Instead, the number of fermentations with at least one commercial yeast strain gives a better picture of the situation as it occurs in vineyards; commercial yeast strains were recovered in 12% of samples.

The global data reflect very different situations. In four vineyards where the sampling sites were placed at a greater distance from the winery, i.e., vineyard F in Portugal and the three French vineyards (A, B, C), the occurrence of commercial yeast was very low, representing between 0% and 2% of the fermentative community, and these strains were isolated from only five samples (Table 2). In France

the genetic profile of 16 clones out of 735 *Saccharomyces* isolates (2%) was identical to that of commercial yeasts. These strains correspond to 0.8% of the yeast strains isolated after fermentation. With only one exception, these strains (15 isolates) had a profile identical to that of the autochthonous strain ICV D254 and were found in the same site (winery B), in pre-harvest samples taken in 2001. This fact could indicate previous dissemination, but it cannot be confirmed since the commercial yeast strain ICV D254 was initially isolated from the same region of the south of France where the study was carried out. One colony was isolated in 2003 in winery C, which had the same profile as K1M ICV-INRA, used in the three French wineries for the last five to 15 years. It is noteworthy that this yeast, which has been used extensively for a considerable length of time, has never been found in the vineyard, except in this case. In the Portuguese winery F, only two isolates with the same profile as the extensively used commercial yeast Zymaflore VL1, in use for five years, were found. The results were very different in the Portuguese wineries D and E, for which a high number of commercial strains was isolated representing 43% and 10% of the fermentative yeast community respectively.

FIGURE 1. Overall (three-year) distribution of commercial yeast strains according to the distance from the wineries in pre-harvest (a) and in post-harvest (b) campaigns

An overview of the dissemination of commercial strains in relation to their distance from the winery is shown in Fig. 2. Ninety-four percent of commercial strains were found in a radius of around 10-200 m from the winery and a large majority (78%) were recovered in sites at very close proximity (10-50 m) to the wineries (vineyards D and E). A major proportion (73%) was collected in post-harvest campaigns indicating immediate dissemination.

The evolution of the total yeast community isolated after fermentation in the different wineries of France and Portugal during the three years studied is shown in Fig. 2. In large part, commercial strains were found in post-harvest samples, indicating immediate dissemination (also shown in Fig. 1). The 296 strains collected had a genetic profile identical to only nine commercial yeast strains from a total of 34 strains used in the six wineries. Although the industrial yeast strains most commonly used in the wineries were usually collected in great abundance in the vineyard, no strict correlation between the utilization level and the frequency of dissemination was evidenced. For example, the strain K1M ICV-INRA was the most widely used in the three French wineries and only one isolate out of 2,160 isolates collected in France had a genetic pattern identical to this strain.

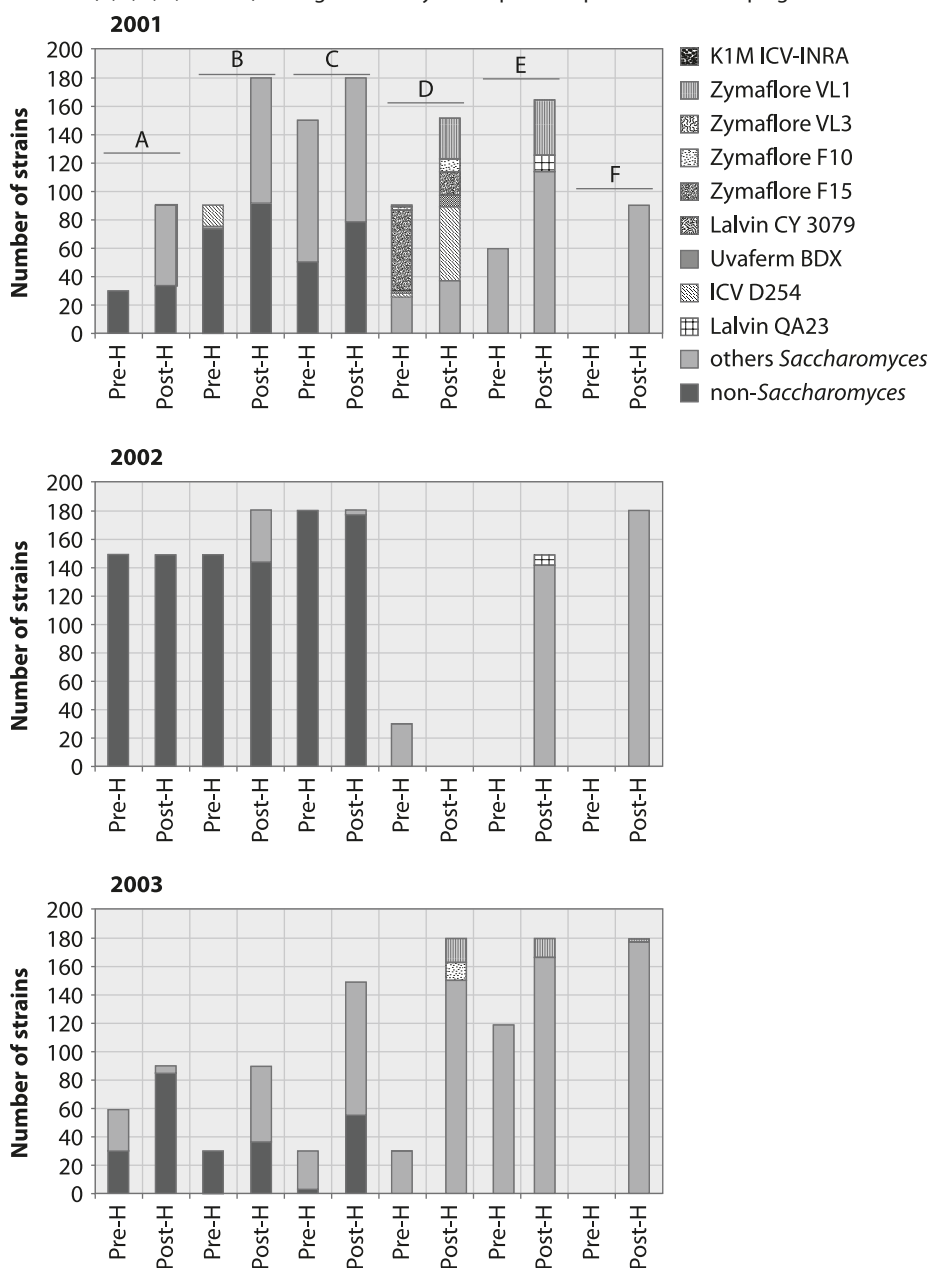
On the whole, the evolution of the fermentative yeast communities over the three years studied showed that the same strains were not found in the same sites from one year to the next. This indicates that if some of these strains are able to remain in the ecosystem, as suggested by the presence of commercial yeasts in pre-harvest samples taken in 2001 in Portugal, they are not capable of dominating the natural yeast community of the vineyard. For example, five different commercial yeast strains were found in the pre-harvest campaign of winery D in 2001, namely the predominantly used strains VL1, F10 and F15 and, in much smaller quantities, the strains Uvaferm

BDX and ICV D254, used from 1998 to 2000, thus showing their survival in the vineyard from one year to the next. However, given that the latter two strains appeared in 2001 only, their permanence is limited.

Conclusion

This systematic study has provided new insights into the impact of commercial yeasts on the communities of fermentative yeasts that inhabit the area surrounding vineyards. The methodology used, based on analysis of the yeast community after spontaneous fermentation, permitted the isolation of a very large number of *Saccharomyces* wine yeasts, which are found in low numbers on the

FIGURE 2. Evolution of the total fermentative yeast communities from each of the wineries (A, B, C, D, E and F) during the three years in pre- and post-harvest campaigns



grapes. It is important to mention that among the 30 colonies analyzed per fermentation, the number of different genetic profiles varied from one to 21, with an average of about five different *Saccharomyces* biotypes per sample (Schuller et al. 2005; unpublished data), indicating that the number of colonies analyzed per sample was high enough to show the initial biodiversity.

Based on these data, we conclude that the dissemination of commercial yeasts in the vineyard is restricted to short distances and limited periods of time. More than 90% of commercial yeasts were found in a radius from 10 to 200 m from the winery and did not become implanted in the ecosystem in a systematic way. Dispersal of commercial strains seems to be mainly mediated by water runoff and occurs also from macerated grape skin at dumping sites. Given that they are used in large quantities, commercial strains tend to out-compete autochthonous strains inside the winery (Beltran et al. 2002). In contrast, they do not seem to settle in the vineyard. Rather, they show natural fluctuations of periodical appearance and disappearance just like autochthonous strains. Considering commercial yeast strains as an appropriate model system for genetically modified yeast strains, our data also contribute to the in-depth environmental risk assessment concerning the use of such strains in the wine industry.

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THE POTENTIAL OF *SACCHAROMYCES CEREVISIAE* WINE YEAST TO IMPROVE RED WINE COLOUR

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The colour of a red wine, when first poured into a glass, provides an important initial impression. For some, the colour gives an expectation as to how the wine will taste. Is the wine deep in colour and hence rich in flavour? Does the wine show a hint of brownness, suggesting that it might be old or tired rather than young and fresh? For the winemaker, is the colour consistent with the style of wine? What are the options for improving the colour? There are a variety of viticultural and winemaking options for modifying the colour properties of wine, however, this article will only briefly address the role of yeast in influencing the depth of colour in Shiraz wine.

Few would dispute that the key element to red winemaking resides with the quality of fruit, and that there is a close link between wine colour and wine quality (Somers and Evans, 1974; Gishen et al. 2002). Much research has been dedicated to understanding the chemistry of grape pigments so that management tools and strategies can be developed to assist in the growing of grapes and production of wine for optimum colour.

The colour of young red wine is largely determined by the phenolic composition, particularly the red coloured monomeric anthocyanins, which are extracted from the grapes into the wine during maceration and fermentation on skins. The colour properties of the anthocyanins are strongly influenced by several factors, but especially wine pH and sulfur dioxide (SO₂) content. At lower pH, higher concentrations of the coloured forms of anthocyanins are present and changes in pH affect the red/purple colour balance, while free SO₂ results in bleaching of anthocyanins (Mazza, 1995). During fermentation and as the wine

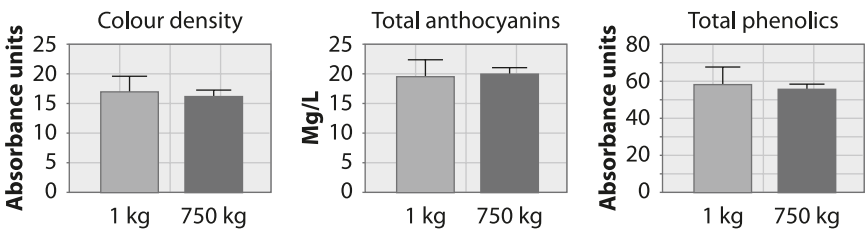
ages, the anthocyanins form more stable pigments by reactions with fermentation metabolites, and by combining with each other and with other phenolic compounds. Thus, only a small proportion of grape anthocyanins that have been extracted from skins during fermentation can be detected in aged red wines, even though the colour intensity is largely maintained (Somers and Evans, 1977; Peng et al. 2002). There are two types of anthocyanin-derived pigments that are important for wine colour; polymeric pigments, which are a heterogeneous group of macromolecules formed by the condensation of anthocyanins with other grape-derived polyphenols, such as tannins, and pyranoanthocyanins and vitisins, which are anthocyanins that have combined with vinylphenols or carbonyls, such as acetaldehyde (Somers, 1966; Fulcrand et al. 1998; Hayasaka and Asenstorfer, 2002; Håkansson et al. 2003). These wine pigments are less affected by pH and SO₂.

The interaction that yeast has with the pigmented phenolic compounds, and hence the impact on wine colour, is not well understood. Current research suggests several mechanisms, including adsorption of pigments to the yeast cell, reaction with yeast metabolites and enzymatic modifications. Recent work with *Saccharomyces bayanus* (AWRI 1375 and AWRI 1176), undertaken at the AWRI, has shown that yeast can markedly affect the colour of young red wine and this effect persists with ageing (Eglinton et al. 2003; Eglinton et al. 2004). However, little is known about the ability of commercial *Saccharomyces cerevisiae* strains to influence wine colour. This topic has recently received attention through a collaborative project between the AWRI Wine Microbiology Team and Lallemend.

The phenolic components (anthocyanins and tannins) that contribute to red wine colour are located in the skin of the grapes. Crushing initiates the liberation of these phenolic components, which is further assisted by the physical and chemical processes that occur during fermentation. In addition to the maceration and extraction effects of carbon dioxide and alcohol formed during fermentation, several yeast-derived metabolites, including acetaldehyde and pyruvic acid, interact with anthocyanins and tannins to form more complex coloured components, such as pyranoanthocyanins and pigmented polymers (Romero and Bakker, 1999). Thus, red wine colour is influenced not only by grape variety, viticultural management practices, geographical growing location, but also by the yeast and fermentation parameters, such as temperature, pH, cap-skin-wine contact management and duration of fermentation.

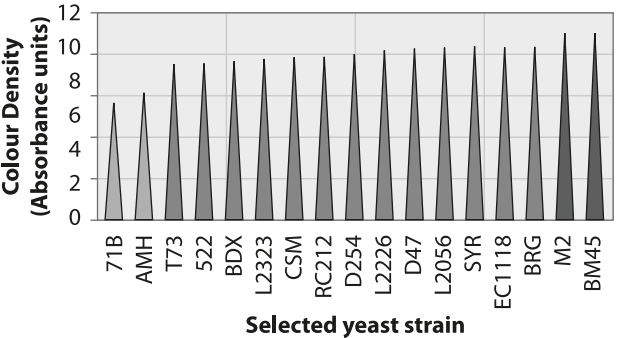
In order to facilitate investigation of the interactions between yeast and the grape must/wine colour components, we have developed a microscale fermentation system that typically uses one kg of grape berries. The iconic Australian red grape variety, Shiraz, has been selected for our yeast fermentation trials. The microscale fermentation system has facilitated a more convenient and rapid screen of numerous commercial wine yeast strains in Shiraz grape musts. The method is robust with little variation between replicates for the typical fermentation parameters and wine colour and phenolics composition. Furthermore, under appropriate conditions this microscale fermentation system produces wines with colour properties and phenolic content comparable to those produced in rotary fermentors on a pilot scale in the Hickinbotham Roseworthy Wine Science Laboratory (see Figure 1). The wine colour density was comparable between the 1kg and 750kg ferments (average 17.6 absorbance units [AU] in the 1kg ferments compared to 17.0AU in the 750kg ferments). Congruent with the wine colour, the total anthocyanin concentration and data for total phenolics of both wines were similar.

FIGURE 1. Comparison of colour and phenolics profile of Cabernet Sauvignon (Padthaway, 2002) wines, at four weeks post-alcoholic fermentation, prepared either on microscale (1kg) or pilot scale (750kg) using *Saccharomyces cerevisiae* strain Lalvin EC1118 to induce alcoholic fermentation. Colour density, total anthocyanins and total phenolics were determined spectrophotometrically (Somers and Evans, 1977; Iland et al. 2000).



Using the microscale fermentation methodology, 17 *S. cerevisiae* strains from Lallemend were screened for effect on the colour of young Shiraz wine (see Figure 2). The four to six-week-old Shiraz wines varied up to 38% in colour density (6.8- 11.0AU). On the basis of wine colour density, the 17 yeast strains could be divided into three statistically distinct groups, which appear to be consistent with industry observations. The differences in wine colour could be easily distinguished by eye. Those wines with the highest wine colour density generally had the lowest wine hue and thus brownness was not a contributing factor to the high depth of colour. The phenolic content of the wines, including malvidin-3-glucoside (the major anthocyanin in grapes), pigmented polymers and tannins, reflected the depth of wine colour. That is, those wines with higher colour density also exhibited higher malvidin-3-glucoside and pigmented polymer concentration.

FIGURE 2. Wine colour density, four weeks post-alcoholic fermentation, of wines prepared with Shiraz fruit (Clare Valley, 2000) and 17 *Saccharomyces cerevisiae* strains on microscale (1kg of fruit).

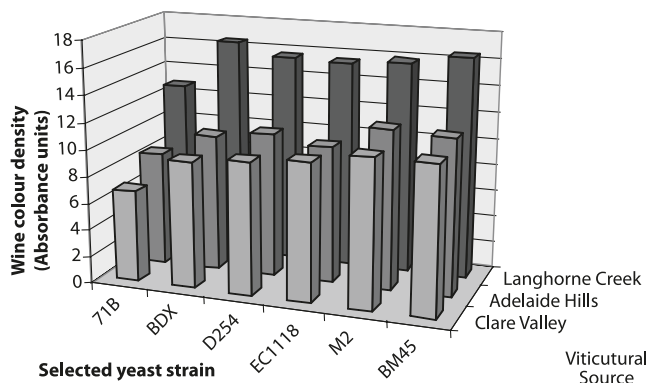


A subset of six yeast strains was selected from the original 17 yeast screened, representing examples from each of the three yeast-wine 'colour' groupings, and used to ferment Shiraz grapes sourced from three different viticultural regions (Adelaide Hills, Clare Valley and Langhorne Creek) (see Figure 3). Interestingly, the yeast strains behaved in an analogous manner for each of these three Shiraz grape musts. The yeast strains that produced wines with a low, moderate or high colour density in the initial 17 yeast screening trial retained this characteristic when fermenting Shiraz grape must from divergent climatic, viticultural regions. That is, wines made with the same yeast strain showed similar relative colour density ranking, irrespective of the source of fruit. Thus, in familiar European terms, the choice of yeast does not mask the effect of *terroir*. Generally, wines vinified with yeast strain 71B tended to contain less of the major pigments (malvidin-3-

glucoside and pigmented polymers) whereas strain BM45 wines tended to contain more. Research currently in progress is suggesting that the relative colour density of young wines is maintained, at least up to eight months, being the last time point measured to date. Thus, the relative colour density of young wines made by the microscale methodology could be indicative of the properties of older wine, with the obvious proviso that other treatments, such as barrel ageing and microoxygenation could confound the effect.

This work, which has been conducted mainly in the laboratory, shows that there is a significant interaction between *S. cerevisiae* yeast strain and the fruit source that impacts on the wine colour density. The relative impact of yeast strain on the wine colour and phenolic content seems to be similar, regardless of fruit source, that is, terroir is preserved. These findings, which still need to be confirmed with other varieties and on a large production scale, suggest that the choice of yeast can be relatively important when maximising colour is needed. It should be remembered, however, that other winemaking techniques, such as the cap management regime, can have a great impact on wine colour as well. Furthermore, research in progress is showing that the choice of yeast strain, and indeed malolactic bacterial strains, can also affect other properties of red wine. Nevertheless, a better understanding of the complex interaction between yeast and grape/wine phenolics will lead to the more efficient use of existing strains and, in the longer term, could lead to the development of improved yeast giving superior wine sensory properties (Eglinton et al. 2004). This is the aim of current research being conducted at the AWRI.

FIGURE 3. Wine colour density, four weeks post-alcoholic fermentation, of wines prepared with Shiraz fruit from three different viticultural regions and six *Saccharomyces cerevisiae* strains.



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THE IMPACT OF SELECTED NATURAL YEAST ON WINE STYLE AND MARKETABILITY

ROUND TABLE DISCUSSION

THE IMPACT OF SELECTED NATURAL YEAST ON WINE STYLE AND MARKETABILITY ROUND TABLE DISCUSSION

Moderator: Joe Wadsack

Wine Writer and Broadcaster, United Kingdom

Panel

Peter Bell: Winemaker, Fox Run Vineyards, Finger Lakes, New York, United States

Eduardo Casadamón: Winemaker, Penaflo Winery, Argentina

Christophe Coupez: Director CEIOE, Bordeaux, France

Carmine Deiure: Winemaker, Contina Tollo, Italy

Pieter Ferreira: Winemaker, Robertson Cellars, South Africa

Daniel Granès: Director of Research, ICV Montpellier, France

Jesús Madrazo: Winemaker, Contino, La Rioja, Spain

Jon McPherson: Winemaker, South Coast Winery, Temecula, California, United States

Hermann Mengler: Dipl.-Ing. (oen.), Head of oenology at the Bezirk Unterfranken, Würzburg, Germany

Kevin Miller: Penfolds Wines, Australia

Following the enlightening presentations by the various scientists at the *XVII^{es} Entretiens Scientifiques Lallemant* scientific meeting, a round table discussion was held with 10 winemakers and oenologists from around the world discussing issues related to the use of naturally selected yeasts and moderated by Joe Wadsack, a British wine personality. Under his professional direction, the participants each briefly described their point of view. The winemakers were representative of different countries, sizes of winery, training and experience. Two of the guest panellists are also involved in consulting centres in two important wine regions of France, Bordeaux and Languedoc, and deal daily with winemakers to advise them on their practices, sometimes helping them evolve into more modern practices to enable them to compete in today's market. All the panellists have two common goals: to make quality wines and to compete successfully in a very difficult world wine market. They addressed such questions as: Can naturally selected yeasts be part of the key to success? Do they feel yeast threatens to generate a uniform wine market where California Chardonnay will taste like Burgundy Chardonnay, and vice versa? What follows is a summary of their discussions.

Wine-style definition and wine yeast – a good combination?

The participants all use natural selected wine yeast, considering selected yeast necessary not only to start alcoholic fermentation but to define the wine style as well. The choice of yeast strain is based on a variety of factors: the quality of the grape, the varietal, the wine style desired and their past experiences from previous vintages.

Peter Bell (New York State), for example, does not like un-inoculated wines. He likes the purity of the flavours and fruit, and that is foremost in his mind. He feels some of the flavours that come out of un-inoculated wines are disturbing and mask the wine's full potential.

Jon McPherson (California) feels that un-inoculated fermentations do not have the consistency he desires when he brings his wines to the market. "Not only is yeast strain selection important," he says, "but so is their nutrition, which is also related to vineyard management and site." As McPherson comes from a region of California less known than other winemaking regions of California, when he makes a Chardonnay, he wants to have the option of enhancing the varietal character, defining his wine

style and producing his Chardonnay, not a Burgundy or Napa Valley Chardonnay.

Pieter Ferreira (South Africa) echoed his concern, saying that he truly believes the use of selected natural yeast helps him maintain his wine style. He can rely on selected yeast for consistency, a key objective when he brings his wines to market.

Kevin Miller (Australia) likes to play with different yeast strains, and he also likes some of the impact a spontaneous fermentation has, especially on texture and viscosity.

Daniel Granès (France) made an interesting parallel. When you make red wines, you could choose different varieties to make a certain style of wine, to define the type of wine you want on the market, based on the maturity of the grapes, the climate, etc. The same holds true when you choose one or several yeast strains to ferment the musts. In France, blending of different tanks is a normal step to get consistent and good quality wine. If you use selected yeast strains, you can take advantage of their different contributions to the wines to eventually define your style through blending.

Christophe Coupez (France) echoed some of the thoughts of his colleague. "Yeast is a tool," he said. He went on to relate that when he first arrived in the Bordeaux region, the winemakers would use yeasts that were as "neutral" as possible, and thought that by doing so, they would respect the quality of the raw material, the grapes. Coupez has been trying to show them that by using high-quality selected yeasts, you will preserve and express the quality of the grapes. Some consultants still think that spontaneous fermentation is the only way to make wine that preserves *terroir* expression. What they don't understand is that the yeast cannot destroy the *terroir* expression any more than stainless steel tanks instead of the old concrete tanks can. He said everyone recognizes the problem of *Brettanomyces*-related odours in some wines, but they do not always understand that by using selected yeasts, they could avoid very undesirable wine aroma compounds.

Eduardo Casadamón (Argentina) explained the situation in his particular setting. At Penaflores they produce different types of wines, have different types of vineyards and grapes are often at very high maturity. They look for a specific yeast strain to complete fermentation. In some neutral varieties they like to use strains that will express more aromas and more complexity, which could not be easily obtained through spontaneous fermentation. Based on the presentations by the scientists at this meeting, he feels that it would be very interesting for wineries to see these results, because it would give them tools to understand

and apply those results. When, for example, they have Sauvignon blanc grapes coming from the same vineyards, they could use several different yeast strains to enhance the complexity of the wine.

Can selected yeast enhance the *terroir*?

Jesús Madrazo (Spain) feels that there has to be different approaches, because it all depends on the wines. If he uses selected yeast strains, will he lose the Contino *terroir* influence? Nowadays, and in years when particular climate conditions exist (in the heat wave of 2003, for example), he uses selected yeasts because the quality of the grapes is unusual. He is starting to understand more about the contribution of yeast to wine. With a new selection (ST7 yeast isolated from Graciano grapes in La Rioja, in collaboration with the University of Madrid), Madrazo is more comfortable using a Contino yeast in order to maintain his typicity. "Although it might be a romantic idea," he said.

Sam Harrop, formerly a wine technologist at Marks & Spencer and now a private consultant, told the group that there are many aspects to typicity. It can be aromas of animal farmyard, due to the actions of *Brettanomyces*. But it can also be, for example, the mercaptans in Chablis that define the typicity of the Chablis *terroir*. Such reductive elements define it – and yeast is necessary for the expression of this *terroir*.

Christophe Coupez asked, "Where is the *terroir* expression potential? It is in the grapes, of course. Selected yeast will preserve and even enhance the *terroir* expression of the grapes and, very importantly, prevent aroma alterations. Ultimately, you don't want any aroma molecules produced by a spontaneous ferment to inhibit the fruit in the wine."

Dr. Paul Henschke commented that a lot of research has been done on flavour precursors, and we seen some of the results during this meeting. We know that yeasts are transformation creatures which use those precursors to transform them into aroma compounds. There are different levels of precursors in each region, within the same vineyards, within the same varietal. Yeasts will release those compounds based on its specific ability to do so, and that varies from strain to strain. Whether you want to call it *terroir* or not, it's up to you! At the AWRI, they ran a yeast trial on colour to see if yeast adds different abilities to express colour. They found that yeast strains have different impacts on colour, and they also saw that yeast preserves the colour characteristics of the different regions they tested, but each strain was different in its ability to

do so. Nutriment also plays an important role. Some yeast strains will not work well in some Australian regions, while others do. There is a strong trend to do regional or *terroir* selection, so that the yeast strain is being matched to the grapes. For example, in low nitrogen regions, they need a yeast strain that can deal with the situation, and if you select a yeast strain locally it will be able to, plus it will produce the flavours of that region and adapted to the product.

Daniel Granès reminded those present that yeast is also a main source of the mannoproteins and polysaccharides that interact with tannins and aroma compounds, as was mentioned during the talks of Dr. Michel Feuillat and Dr. Henschke. The quality of mannoproteins varies from one strain to another and that will influence the texture, the colour stability and the aroma.

Dominique Delteil, formerly at the ICV and now a private consultant, said that the *terroir* is such a general concept, and relates not only to the soil or the climate. As some *terroirs* are not always so nice, respecting the *terroir* might not be so exciting, but sometimes using the right yeast can improve it.

Can the value of wine be increased through proper fermentation management?

Hermann Mengler (Germany) wants to make good wines and, yes, he wants to make money selling his wines. To do this, he has to make good wines all the time, and if the consumer likes it, then the goal has been reached. In Franconia, they have a wine "pyramid." At the bottom of pyramid is the "normal" wine, very fruity and crisp with 11.5-12% alcohol, positioned as an easy-drinking product, and they use an all-around yeast. In the middle of the pyramid is wine that has a longer life span (one to three years) and he prefers using a strain selected from a region that will complement the wine style he is seeking or, even better, a strain from a particular region. At the top of the pyramid, the wines are designed more for aging, and you can almost taste the soil, the valley, the *terroir*. It is a full-bodied wine, with concentrated flavour. The winemaker will ask, "What is the risk associated with a spontaneous fermentation?" If the fruit is beautiful, they will often allow spontaneous fermentation to occur.

Kevin Miller is an advocate for selected yeast and will use from 10 to 12 different strains. In premium wines, he is less willing to take risks and his philosophy is to maintain wine quality. Pieter Ferreira commented that there is also the question of climate. In a sunny region, where

wines have high alcohol potential, selected yeast strains are needed to complete fermentation. In both South Africa and Australia, the fruit needs to ripen for a long period in order to get all the physiological factors in balance, and that often means high sugar musts.

Carmine Deiure (Italy) reminds us that the final taste of the wine has one objective – to please the consumer, whether or not the consumer is a professional wine drinker. The wine needs colour and flavour, and the winemaker can choose a selected yeast strain to develop the flavour of the wine.

In Languedoc, according to Daniel Granès, selected yeast strains are used for table wine without much thought given to using the proper strain. For premium wines, it might take a few years of trial and error, changing the strains to find the best combination.

With the new labelling laws in many countries, there is a lot of concern regarding SO₂, biogenic amines and ochratoxin A reduction. If a selected yeast strain can help with the reduction of those compounds, would it not be interesting to winemakers? As Jon McPherson pointed out, it is definitely a point that needs to be addressed.

Are selected yeast strains as romantic as spontaneous ferments?

Christophe Coupez knows that there is some opposition to the use of selected yeast strains, and the decision to use them is related to the target market you are aiming for. He is wondering why people think that spontaneous fermentation can add something more than selected yeast strains used intelligently. For him, spontaneous fermentation is a gamble, and if off-aromas develop, you lose a lot of effort, money and eventually a great product.

Cornelius Van Casteran, a Dutch wine journalist, related the following anecdote. Some consultants are advising some very renowned classic *châteaux* and cellars, and they are strongly advocating the use of spontaneous fermentation. However, the winemakers do not like having to risk stuck fermentation, and if they see the volatile acidity rising dangerously in the premium *cuvées*, they re-evaluate their fermentation strategy to increase their chances of having quality and consistency in their products.

McPherson laughingly added that spontaneous fermentation is a good thing for companies that specialize in removing volatile acidity from the wines.

Coupez went on to say that a lot of big *châteaux* are using selected yeast, but don't want that fact to be known outside the premises. The use of selected yeast is gen-

eralized in Bordeaux, but the claim of spontaneous fermentation is still going strong. Madrazo added that visitors coming to the winery could think that selected yeast strains are chemicals, so they usually avoid saying anything about the winemaking, particularly regarding yeast and bacteria.

Dr. Henschke wonders why people are so fixated on spontaneous fermentation. He reminded those in attendance that according to Dr. Sylvie Dequin's presentation, the resident yeasts are usually in the field, not even on the grapes, so how can you say that the best yeast for a particular *terroir* or winery is the wild one, as this yeast has probably never touched a grape berry before? According to him, using a spontaneous fermentation is like going to the races and not knowing which horses are running: you bet your money, and only luck decides if you win or lose. Along with the winemakers in Australia, he is wondering what they are getting in term of objectives that drives them to use that system. Miller answered by saying that he feels spontaneous fermentation will give your wine more mouthfeel and texture and some controlled sulphide character.

As Claude Espeillac, director of the fermented beverage group at Lallemand, reminded those present, selected yeast is in fact the best spontaneous strain from a particular *cuvée*.

According to Granès, you have to keep in mind that it isn't a good idea to think in terms of one strain of yeast only, but more in terms of a blend of strains in different tanks.

Pieter Ferreira mentioned that when you start picking the grapes, you start a spontaneous fermentation, since there might be a three-to-six-hour waiting period between the field and the winery before the grapes are processed. Peter Bell added that at the beginning of fermentation there are other microorganisms at work, but at the end of fermentation, where it gets to the crucial part and you do not want any residual sugars, the selected yeasts are predominant.

Granès confirmed that during a study done at the ICV many factors were involved in the input of spontaneous ferments before the onset of fermentation with selected yeasts. The sanitary conditions, the time between picking and processing, the management of SO₂ – all contribute to the equilibrium, in that case, with ICV-INRA K1, and the other microorganisms. That equilibrium can change depending on the state of all those factors. Dominique Delteil confirmed that the inoculation rate will also influence the amount of spontaneous ferment carrying on in the fermentation.

Is there a need for genetically modified yeast strains?

The discussions took an interesting turn as Daniel Granès commented that the presentation of Dr. Florian Bauer was very interesting, but he was wondering why there would ever be a need for GM yeast? Dr. Bauer answered that there is not a lot of genetic stability in nature. Granès replied that he has a different opinion since he has seen a lot of stability in their strains, and within strains, a very different contribution to the wines. "I am concerned about having one perfect strain of yeast," he explained. "The yeast you choose is usually picked the day before you plan to use it and it is based on the grape quality. We have a very large range of different yeast strains available and maybe a GM strain can enlarge that range, but for now, it is interesting and wide enough and the differences between the strains are also large enough." Dr. Bauer agreed that GM yeasts are not a silver bullet and won't be able to solve everything, but they can add another aspect to the winemaking possibilities. In the future, markets, wine and health will be interesting topics to discuss and could be an opportunity to look at GMOs. But consumers, particularly in Europe, are scared of these microorganisms.

Gerd Steep, wine technologist at Marks & Spencer in the United Kingdom, feels that there is a very low level of acceptance for GMOs, even in the U.K., and especially in wine. GMOs were successfully introduced into other products, but wine has an image of nature and purity and it would be very difficult to introduce them.

One important issue is the fact that selected or spontaneous yeasts cannot deal with every situation. One of the most-asked questions was regarding high alcohol potential in some warm climate regions that can be a real challenge. Do scientists think it is feasible to have a natural selected yeast strain that would have a low sugar-to-alcohol conversion, or would a GM yeast strain be the only answer? Dr. Henschke feels that there are other alternatives, such as non-*Saccharomyces* yeasts, which on their own can not complete fermentation, but only through sequential inoculation (non-*Saccharomyces* at the beginning to degrade some of the sugars, followed by a strain of *Saccharomyces* to finish the fermentation). But in many cases, the reduction in ethanol content is not enough. Some work was done with GM yeast, but there was a downside to this research: the ethanol was reduced, but the conversion pathway was changed and there was an increase in volatile acidity production. The yeast system is complex and if you change its metabolism, there are risks that other compounds might be overproduced.

Sam Harrop added that it is important to understand that we are putting the diversity and typicity, not only the wine style, at risk if GMOs are used in this market. GMOs can do wonderful things, but we have a responsibility to maintain tradition. In order to have a sustainable industry, some key factors such as diversity and the natural side of fermentation need to be kept in mind. On this point, Dr. Bauer does not agree. GMOs can add to diversity, they are not monsters and are also natural. It is all an issue of perception, he said.

Steep added that, as a wine retailer, the word "natural" is important for consumers and that the use of GMOs is too difficult and controversial for them, and that it is too early for GMO wines in the United Kingdom.

There is now a GMO yeast strain available in the United States, but it has already been banned in Sonoma County. Gordon Specht, the U.S. market manager at Lallemant, said that winemakers look interested, but if they do use this GMO they will not make any noise about it.

Dr. Dequin continued on this topic by saying that the reduction of alcohol content in wine is a real challenge and so far, with what we have in nature, it has been impossible to do reduce alcohol with *Saccharomyces cerevisiae*. You then have to look at alternative approaches, such as developing new yeast strains by adaptation, mimicking what nature is doing, or modifying yeast metabolism. One way or another, the solution to this problem will be very different from what we are actually doing. For example, when you need to reduce alcohol level up to 2%, the sugar must be converted into something else, such as glycerol, but it would not be sufficient and other compounds would be produced, such as acetic acid and other metabolites. If there were a property that would need to be improved via a GMO, it would have to be a strain with a low ethanol yield.

Granès feels that the solution is not only in the winemaking, but, very importantly, in the viticulture. In some research being done in Languedoc, they are looking at the maturity level, where you usually have to wait until reaching a 13% alcohol potential to get the phenolic maturity. Instead of finding GM yeast, it might be easier to have better vineyard management.

Dr. Henschke asked, "If you could find an extraordinary application for GM yeast, like, for example, a yeast strain with increased resveratrol that would extend your life five years, would it be more acceptable?"

Is the perception of selected yeast mistaken?

Dr. Bauer pointed out that he felt some winemakers seemed kind of apologetic about using selected yeast. For him, wine is a modified product: grapes transformed into wine through the action of yeast. You need yeast in order to have wine. Kevin Miller answered that he is not apologetic about using selected yeast. Penfolds is a traditional institution, but their style is innovative and central to their philosophy. They are not afraid to announce that they are using selected yeast or even which yeasts they are using.

Granès said that wine is the meeting between grapes and yeast, but with a recent communication they realized that a lot of people lack the culture related to the technical aspects of winemaking. On top of that, since yeasts are microscopic organisms, most people think that they are actually dangerous. There is a definite need to build a "microbiological" culture inside and outside the wine industry.

Madrazo also thinks that wine is complex and difficult to understand and agrees that there is a lack of education when it comes to microbiology, although he sees that English-speaking countries are usually more open-minded about this side of winemaking.

Coupez added that often the consumer does not know how wine is made and if it is mentioned that selected yeast are used, it's like saying that it is an industrial process, in the mind of the consumer, that means the wine is being standardized. Those reflections must change in order to show that winemaking is a legitimate process.

Hermann Mengler spoke up, saying that if a wine is produced in the Old World, they usually don't say which yeast strains are being used. In Germany, for example, wine sales are possible only when you target emotions and feelings, and technical aspects do not make wine sales.

Cornelius Van Casteren added another point. In the end, the consumer is looking for a wine that will have good quality year after year. They like to read about those typical wines, but, really, what they look for is the reliability of the brand. There is nothing very sexy about the brand, but they will read about the small typical wineries and then they go buy the big brands.

Winemakers share their secrets

Kevin Miller, at Penfolds in Australia, can use six or seven different yeast strains in white wines and two or three different strains in reds, to match the varietal and the region. Peter Bell, from Fox Run Vineyards in New York State, said

that it depends on the different grape varieties. He has nine or 10 different yeast strains he likes to use and they produce 12 to 14 different wines at the winery. For example, for his Pinot noir, he needs several yeasts in different tanks to make a good wine through blending. Of course, this changes from year to year, depending on the colour.

Jon McPherson, of South Coast Winery in California, explained that it is vintage, variety and market dependant, but he usually uses three to five strains per colour.

Pieter Ferreira, from Robertson Cellars in South Africa, also claimed that it is variety and vintage dependant. He likes to be careful when using so many strains and during the crazy frenzy of harvest time, you have to make sure you inoculate the right yeast strains into the right tank.

Eduardo Casadamón, of Penaflor Winery in Argentina, said that it is variable at each winery, and depends on the winemaker and the winemaking conditions. It is important to have uniform conditions for the grapes because different strains can give different results. They know that only one yeast strain can produce one wine and a combination of several yeasts can help with the quality of the wine.

Daniel Granès, director of the ICV in Montpellier, related that the usual trend in the different wineries in Languedoc is to use three to five strains per colour, depending on the market segment targeted.

Christophe Coupeze, director of research at the CEIOE in Pauillac, thinks that when the wine profile you want is defined, you can select three to five different strains depending on the style you want.

Carmine Deiure, of Contina Tollo in Italy, said they usually have two to four different strains per wine, since the choice is vintage dependant, and also depends on the market segment the wine is for.

Hermann Mengler, from Franken Wines in Germany, told the group that they usually have a choice among 15 to 25

yeasts and the winemakers will choose five to six strains, depending on the wine style and conditions.

Lastly, Jesús Madrazo, from Contino in La Rioja, Spain, will use five different strains and a Contino isolate. His strains include the ICVD80 and ICVD254. At the end of harvest, he will also have tanks with spontaneous fermentation that represent about 30% to 40% of his wine.

Craft, tradition and science

As Joe Wadsack concluded, the meeting was interesting and the discussion vigorous, and there was a surprising amount of consensus among the panellists. One thing for sure, the winemakers are all preoccupied with similar concerns: making quality wine, making distinctive wine, reaching the consumers and selling their products.

"We know that some regions are more linked to tradition than others," he commented, "and that will lead to a 'secret' life when it comes to the use of selected yeasts, as even though selected yeast strains are being used during winemaking, it is a subject not mentioned in order to keep the magic of winemaking intact or to maintain a 'romantic' approach, as one of the winemakers said. The real challenge is to bring a harmonious link between craft, art and science. It is a step-by-step event, and sometimes it might be too fast, as we have seen in the heated debate on GM microorganisms. The key might just be a mix between tradition and science."

At Lallemand, we feel it is our responsibility to communicate to consumers, journalists and winemakers that natural selected yeast is not a synthetic product resulting from an industrial chemical process. In fact, every natural selected yeast strain was first a very good, dominant spontaneous ferment. Using selected yeast instead of spontaneous ferment takes away none of the romance associated with winemaking. Natural selected yeast simply ensures the consistent quality winemakers – and consumers – desire.

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