CHAPTER 9

Wine Microbial Spoilage: Advances in Defects Remediation

Fernanda Cosme, Alice Vilela, Luís Filipe-Ribeiro, António Inês, Fernando M. Nunes

CQ-VR—Chemistry Research Centre, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

1 Introduction

Since microbiological activity can develop quickly and without warning in wine, early identification of possible spoilage problems is critical to implementing corrective remedies. However, identifying the contributing microorganisms and the appropriate correction is not always simple, because a given microorganism can cause multiple spoilage problems, and a specific wine fault can be caused by different microorganisms. The contaminations are often undetected, until the wine problem becomes noticeable by sensory evaluation. Spoilage microorganisms besides affecting the healthiness of wine can make it unsafe for human consumption, by producing biogenic amines (BA) and precursors of ethyl carbamate (Capozzi et al., 2012). A diversity of fungi can infect grapes prior to harvest, the principal ones being species of *Botrytis, Uncinula, Alternaria, Plasmopara, Aspergillus, Penicillium, Rhizopus, Oidium*, and *Cladosporium* (Fleet, 2001). Fungal growth on grapes can produce various metabolites and conditions that may disturb the ecology and growth of yeasts during alcoholic fermentation. There are reports that *Botrytis cinerea*, *Aspergillus* spp., and *Penicillium* spp. produce metabolites that retard the growth of yeasts during fermentation (Doneche, 1993).

In wines, the main spoilage microorganisms are yeasts of the genera *Dekkera/Brettanomyces*, *Candida*, *Hansenula*, *Hanseniaspora/Kloeckera, Pichia*, *Saccharomycodes*, and *Zygosaccharomyces,* among others (Fig. 9.1); the lactic acid bacteria (LAB) *Lactobacillus*, *Leuconostoc*, and *Pediococcus*; and the acetic acid bacterial genera *Acetobacter* and *Gluconobacter* (Du Toit and Pretorius, 2000). The faults caused include excessive bitterness and off-flavors, such as ester taint, vinegar, butter, geranium character, and phenolic (horsy) character (Bartowsky, 2009) (Figs. 9.2 and 9.3). They also cause sensory visual problems, such as film formation, turbidity, viscosity, and sediment (Fig. 9.4). The formation of volatile phenols (4-ethylguaiacol and 4-ethylphenol) (Chatonnet et al., 1997) and mousy off-odor by acetamide production of tetrahydropyridines (Costello

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Spoilage effect	Causal microorganism
Ester and aldehyde taints; increased volatile acidity	*Pichia, Hanseniaspora, Hansenula, Metschnikowia, Dekkera, Candida*
Formation of surface films	*Candida, Pichia*
Mousy, horsy taint	*Brettanomyces*
Refermentation in the bottle	*Saccharomyces, Zygosaccharomyces bailii*
Oxidized taint from acetaldehyde	*Saccharomycodes ludwigii, Pichia membranafaciens*
Deacidification of wine	*Schizosaccharomyces*

Figure 9.1: Yeasts-Related Faults in Wine and Examples of Causal Microorganism.
Adapted from Considine, J.A., Frankish, E., 2014. A Complete Guide to Quality in Small-Scale Winemaking, first ed. Academic Press, Waltham, USA.

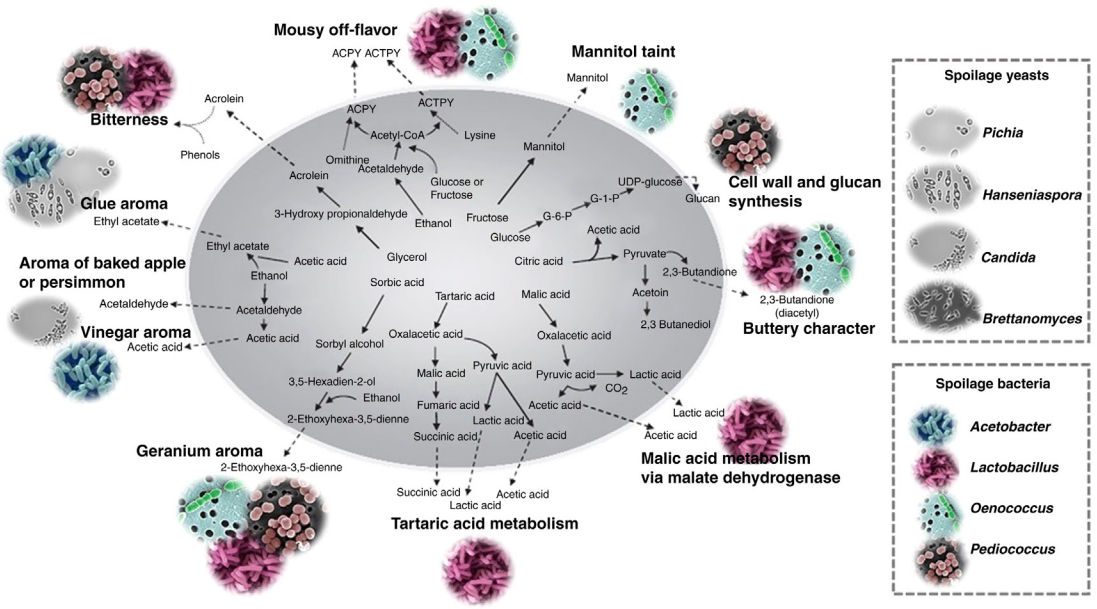


Figure 9.2: Summary of Bacterial and Yeasts Pathways Leading to Spoilage Aroma and Flavor Compounds of Wine.
Adapted from Bartowsky, E.J., 2009. Bacterial spoilage of wine and approaches to minimize it. Lett. Appl. Microbiol. 48 (2), 149–156.

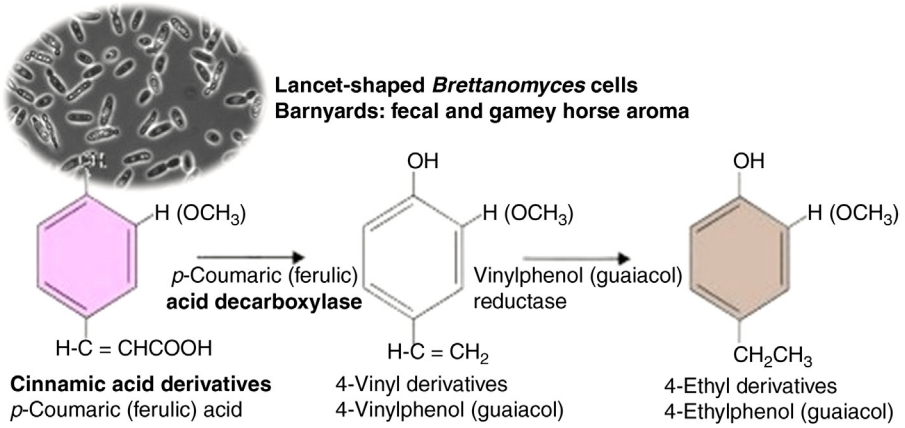


Figure 9.3: Pathway to the Formation of 4-Ethylphenol and 4-Ethylguaiacol in Wine. A carboxylase decarboxylates the hydroxycinnamic acids into the corresponding vinyl derivative (4-vinylphenol from *p*-coumaric acid or 4-vinylguaiacol from ferulic acid). In the second reaction, a reductase converts the vinyl group into the corresponding ethyl compound.

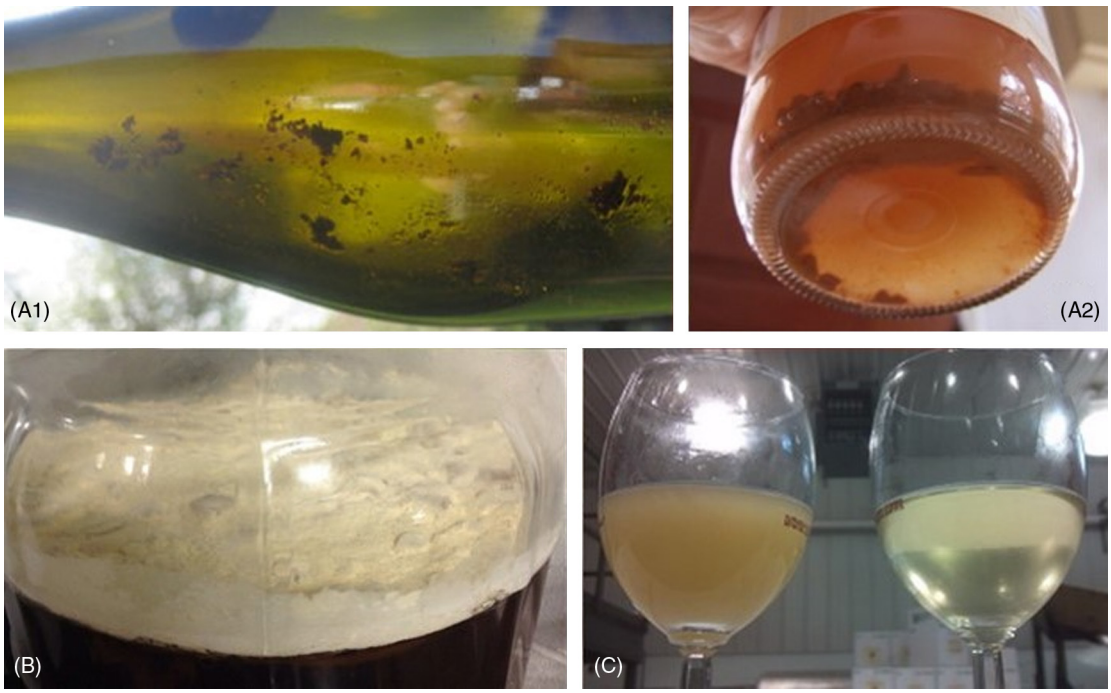


Figure 9.4: Microorganisms sensory visual problems, such as sediment (A1 and A2); film formation (B); and turbidity and viscosity (C).

and Henschke, 2002) during malolactic fermentation (MLF) can also occur. LAB can also be responsible for the production of other compounds, which will impart negative effects on wine quality, such as the formation of ethyl carbamate by the degradation of arginine (Liu, 2002) and BA, such as histamine, tyramine, and putrescine, by the degradation of precursor amino acids (Arena and Manca de Nadra, 2001; Lonvaud-Funel, 2001), compounds that may affect the consumer's health. Although less frequent, in our time, bitterness by acrolein formation from glycerol degradation (Claisse and Lonvaud-Funel, 2001), butter aroma due to excessive production of diacetyl (Martineau and Henick-Kling, 1995), flocculent growth (Amerine and Kunker, 1968), mannitol taint (Sponholz, 1993), ropiness (Walling et al., 2001), tartaric acid degradation (Sponholz, 1993), and the geranium off-odor (Crowell and Guymon, 1975) may occur. Besides bacteria and yeasts, filamentous fungi, such as *Aspergillus,* may likewise be responsible for depreciating the wine quality by the production of mycotoxins, such as aflatoxins (AFs), ochratoxins, and fumonisins, which are also detrimental for human health (Zain, 2011). *Aspergillus* black rot is among one of the many bunch rots occurring on grapes. The disease appears on the berries as a black rot due to prolific fungal sporulation after it has attacked and consumed the berries, which look completely empty and dry. This fungus can be isolated using the Dichloran Rose Bengal Agar medium, a selective isolation and enumeration of fungi-yeasts and molds, of significance in food spoilage medium (Fig. 9.5) (Somma et al., 2012).

The selection of yeast and bacteria strains for performing alcoholic and MLFs, gathering the best enological characteristics and eliminating the bad ones may be the first step for achieving wines with the best quality. Also, ability of wines deacetification, detoxifying mycotoxins, and BA must be additional criteria in yeast and bacteria selection for vinification.

The early detection and identification of wine spoilage microbes can be done by using microbial physiological techniques, including plating in selective and differential media (Kántor and Kačániová, 2015), molecular detection like PCR-based DGGE analysis, and qRT-PCR, among others (Mills et al., 2008), and by metabolomics.

The composition of wine is determined by a complex interplay between environmental factors, genetic factors, and viticulture practices (Roullier-Gall et al., 2014), and metabolomics offers the toolbox for integrated analyses of the wine composition resulting from all of these factors. Metabolomics approaches have shown great potential for the study of grapes and wines (Flamini, 2014). The concept of wine omics for the untargeted wine analysis coupling NMR and GC-MS were followed by the oenomics approaches. All inclusive analysis require access to sensitive and powerful instruments. Therefore, metabolomics employs high-resolution techniques, such as FTICR-MS, LC-MS, GC-MS, and NMR spectroscopy, combined with multivariate statistics (Roullier-Gall et al., 2014).

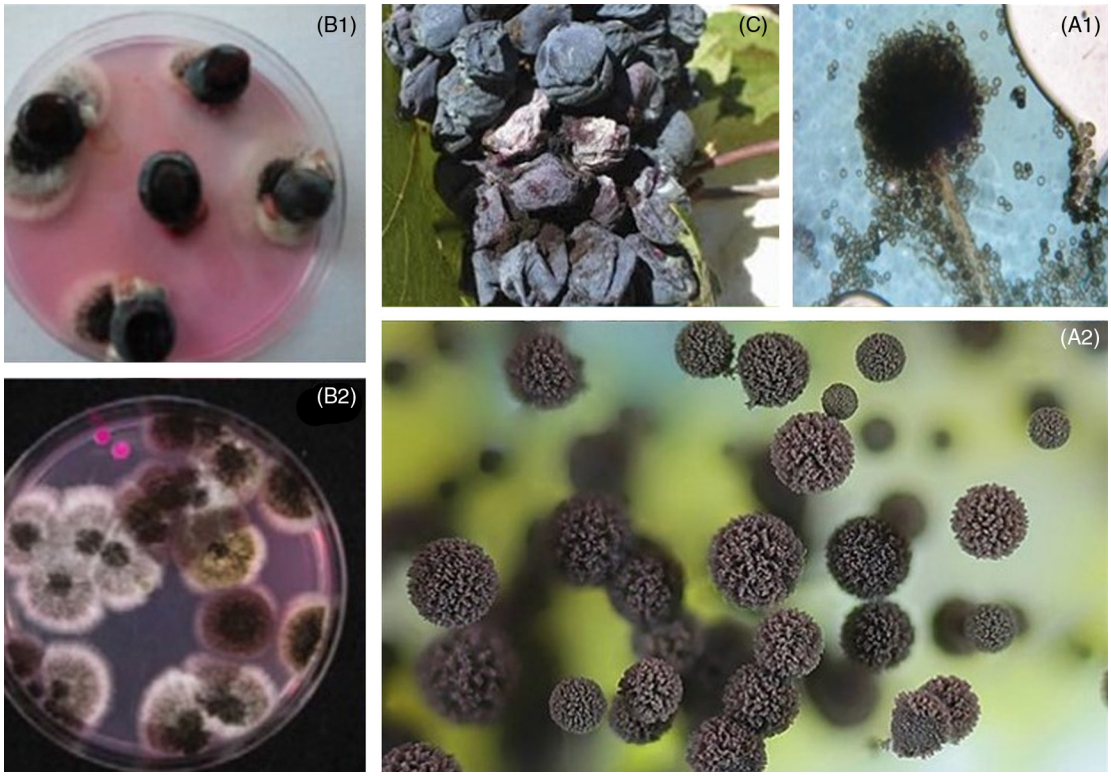


Figure 9.5: *Aspergillus* conidiophore (A1) and conidia (A2). Different black *Aspergillus* colonies from berries from direct plating on Dichloran Rose Bengal Chloramphenicol (DRBC) agar homogenate (B1), diluted and plated on DRBC (B2). Black rot of berries caused by black *Aspergilli* (C). *Adapted from Somma, S., Perrone, G., Logrieco, A.F., 2012. Diversity of black Aspergilli and mycotoxin risks in grape, wine and dried vine fruits—review. Phytopathol. Mediterr. 51 (1), 131−147.*

Metabolomics approaches have been used in different food matrices to identify compounds related to a particular microbial contamination, usually via GC-MS, HPLC, SIFT-MS, and NMR (Fig. 9.6) (Leroy et al., 2014).

2 Wine Microbial Spoilage

2.1 Acidic Wines: Can Wine Turn Into Vinegar?

Over the course of time, numerous winemaking strategies were developed, resulting in a range of wine products, from Champagne to fortified wines like Port wine. However, since the time of Louis Pasteur (around 1873) the microbial contribution to the production of wine has become a subject of research and, often, debate (Mills et al., 2008). Pasteur discovered that beers, wines, and many other fermented products were produced by yeast.

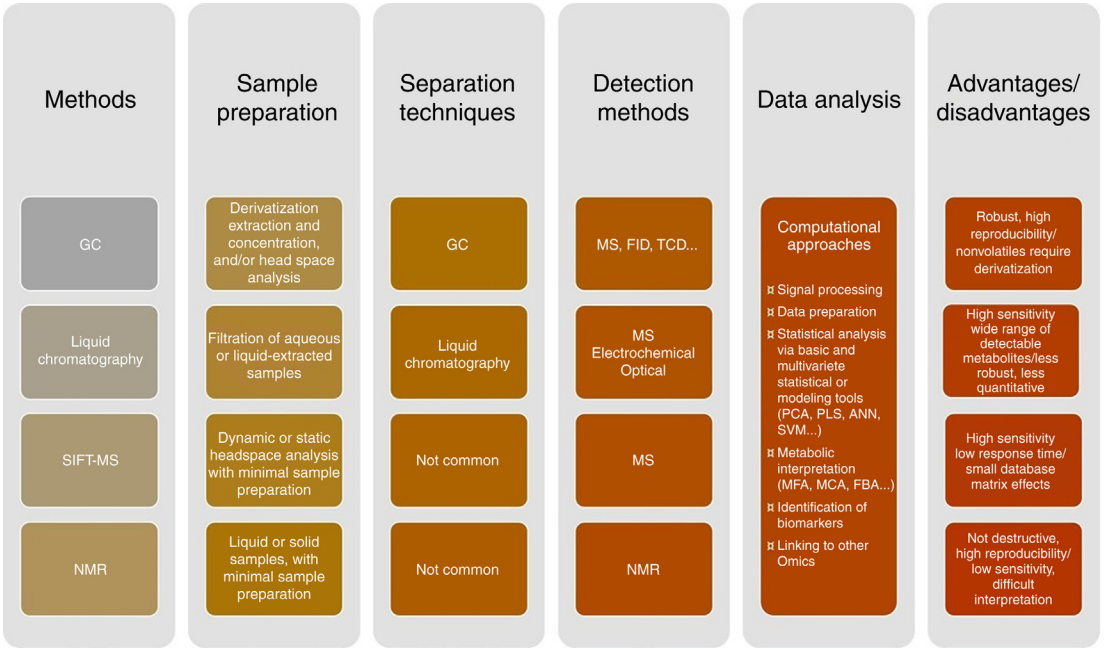


Figure 9.6: Examples of Methods for Metabolic Analysis Aiming at the Establishment and Analysis of Food/Wine Metabolomes.

Adapted from Leroy, F., Kerrebroeck, S.V., Vuyst, L., 2014. Metabolomics. In: Battt, C.A., Tortorello, M.L., (Eds.), Encyclopedia of Food Microbiology, Elsevier, New York, pp. 780–787.

However, when a spoiling effect occurred, Pasteur found that the microorganisms present in the beverage are much smaller than the yeast cells. Pasteur concluded that these smaller microorganisms, called bacteria, caused wine to spoil into a vinegar-like product. Over 150 years later, winemakers still deal with this spoilage in wines today.

Volatile acidity (VA) is derived from acids of the acetic series in the "free state" and combined as salts (Office International de la Vigne et du Vin, 2009). In extreme quantities, volatile acids confer to the wine an unpleasant vinegar aroma/flavor associated with a sour taste. The maximum acceptable limit for VA in most wines is 1.2 g/L of acetic acid (Office International de la Vigne et du Vin, 2010). The aroma threshold for this acid depends on the wine style and variety. An acetic acid concentration of 0.90 g/L can produce a clear bitter and sour aftertaste in wine, still not causing a strong odor (Ribéreau-Gayon et al., 2006a). Acetic acid can be formed at the beginning of wine production (in grapes), during fermentation, and in the bottled wine as a bacterial or yeast metabolite (Table 9.1) (Vilela-Moura et al., 2011).

Bacteria, in *B. cinerea*-infected grapes, can produce acetic acid. Due to rupture of grape berry skin, *B. cinerea* infection permits access of bacteria to the berry's interior. *Acetobacter* species dominate on the surface of rotten grapes. These microorganisms use the ethanol produced by wild yeasts as a carbon source, producing acetic acid (Du Toit and Lambrechts,

Table 9.1: Acetic acid in grapes and wines: microbial origin and range of concentrations.

	Responsible Microorganism				
	Acetic Acid Bacteria	**Filamentous Fungi**	***S. cerevisiae***	**Lactic Acid Bacteria**	**Spoilage Yeast**
Grape musts	• Oxidation of ethanol to acetic acid by *Gluconobacter* spp. and *Acetobacter* spp. in *B. cinerea*-infected grapes (Du Toit and Lambrechts, 2002) • Unspoiled grapes (less than 10^2–10^3 cells/g of *G. oxydans*, contain 0.016–0.023 g/L acetic acid • Spoiled grapes (as high as 10^6 cells/g, mainly *A. pasteurianus* and *A. aceti*) contain up to 3.9 g/L acetic acid (Joyeux et al., 1984a,b)	• *A. niger*, *A. tenuis*, *C. herbarum*, *R. arrhizus*, and *Penicillium* spp., typically associated with sour rot, are responsible for black fungal sporulation and typical sour rot symptoms—berry cracking and leakage along with a pungent vinegar smell (Rooney-Latham et al., 2008) • Musts made with partially sour grapes can contain more than 1.0 g of acetic acid and several grams of gluconic acid per litter (Ribéreau-Gayon et al., 2006b)		• Due to very rapid growth of indigenous *Lactobacilli* (so-called "ferocious") in improperly treated freshly crushed must, which leads to high concentrations of acetic acid, rather than lactic acid (Boulton et al., 1996)	• *Hansenula* spp. in unsulfited and noninoculated must (Ribéreau-Gayon et al., 2006b) • *B. bruxellensis* is also thought to be associated with grape contamination (Zuehlke et al., 2013)
Alcoholic fermentation (AF)	• *Pasteurianus* and *A. liquefaciens* that survive during fermentation can also increase the acetic acid content of wines and may cause wine spoilage (Du Toit and Lambrechts, 2002)		• Healthy grape must with sugar concentration (<220 g/L) can display 0.1–0.3 g/L of acetic acid • Some *S. cerevisiae* strains produce more than 1 g/L of acetic acid (Ribéreau-Gayon et al., 2006b)	• Mainly produced by heterofermentative *Oenococcus* spp., even if there is a small residual sugar concentration. Acetic acid is produced (0.12–0.24 g/L) from sugar after their growth phase associated with MLF (Ribéreau-Gayon et al., 2006b)	• *P. anomala*, *Candida krusei*, and *C. stellata* (Fleet and Heard, 1993), as well as *Hanseniaspora uvarum*/*K. apiculate* (Romano et al., 1992), produce high levels of acetic acid (1–2, 1–1.3, and 1–2.5 g/L, respectively) before and during initial fermentation steps, leading to serious wine deterioration (Fleet and Heard, 1993) • *S. ludwiggii* strains can produce acetic acid concentrations higher than 0.75 g/L (Romano et al., 1999)

(*Continued*)

Table 9.1: Acetic acid in grapes and wines: microbial origin and range of concentrations.

	Responsible Microorganism				
	Acetic Acid Bacteria	**Filamentous Fungi**	***S. cerevisiae***	**Lactic Acid Bacteria**	**Spoilage Yeast**
MFL				• The decarboxylation of malic acid into lactic acid by LAB (*O. oeni* and *L. plantarum*), is associated with changes in the amino acid composition and increment in acetic acid concentration (0.05–0.32 g/L) of the initial VA (Pozo-Bayón et al., 2005)	
Wine	• A substantial increase in acetic acid (0.5–3.5 g/L for 1×10^3–9×10^4 cfu/mL) and a decrease in ethanol concentration was correlated to an increase in *A. pasteurianus* population (Bartowsky and Henschke, 2008)			• *Lactobacillus, Pediococcus,* and *Leuconostoc* spp., almost nondetectable in wine, cause spoilage when growth occurs by producing 0.8–6.1 g/L acetic acid (Sponholz, 1993)	• Species of the genus *Dekkera/Brettanomyces* (Boulton et al., 1996; Pretorius, 2000) produce acetic acid up to 7.2 g/L (Sponholz, 1993)

LAB, Lactic acid bacteria; MLF, malolactic fermentation; VA, volatile acidity.

Source: Adapted from Vilela-Moura, A., Schuller, D., Mendes-Faia, A., Silva, R.F., Chaves, S.R, Sousa, M.J. Côrte-Real, M., 2011. The impact of acetate metabolism on yeast fermentative performance and wine quality: reduction of volatile acidity of grape-musts and wines—minireview. Appl. Microbiol. Biotechnol. 89, 271–280.

2002). Fungi associated with sour rot, such as *Aspergillus niger*, *Alternaria tenuis*, *Cladosporium herbarum*, *Rhizopus arrhizus*, and *Penicillium* spp. can also be responsible for the manifestation of acidic musts, with more than 1.0 g/L of acetic acid (Ribéreau-Gayon et al., 2006b; Rooney-Latham et al., 2008) (Table 9.1).

Saccharomyces cerevisiae produce acetic acid as a byproduct of alcoholic fermentation, mostly at the start of fermentation. This production is dependent on the yeast strain, grape-must composition, and must nitrogen concentration (Delfini and Costa, 1993; Torrens et al., 2008; Vilanova et al., 2007). In response to sugars hyperosmotic stress, yeasts, in wine, produce acetic acid to equilize the redox balance. This phenomenom is particularly severe in grape-must with a ºBrix (sugar content) higher than 35 (Erasmus et al., 2004). Yeasts, such as *Dekkera*/*Brettanomyces* (Sponholz, 1993), and apiculated wine yeasts, such as *Hanseniaspora*/*Kloeckera* (Ciani and Maccarelli, 1998), can produce acetic acid up to 7.2 g/L. At the end of the fermentation process and during wine storage *S. ludwigii*, a spoilage yeast frequently isolated from wine, can also produce detrimental amounts of acetic acid (Romano et al., 1999).

The decarboxylation of malic acid into lactic acid by lactic acid bacteria (BAL) during MLF is associated with changes in the amino acid content of wines, leading to an increase of the initial VA (Pozo-Bayón et al., 2005). Starter cultures of *Oenococcus oeni*, under pantothenic acid deprivation due to CoA deficiency, can produce acetate (Richter et al., 2001). This production is also due to competition between yeasts and bacterial populations during concurrent MLFs (Boulton et al., 1996). Acetic acid bacteria (*Acetobacter pasteurianus* and *A. liquefaciens*) can also increase the acetic acid content of wines and may cause wine spoilage (Du Toit and Lambrechts, 2002). Furthermore, after bottling, red wines under peculiar circumstances, may carry a small population of acetic acid bacteria that proliferates in bottles stored in an upright position (Bartowsky and Henschke, 2008) (Table 9.1).

2.2 Ester Taint: Banana or Glue?

Yeasts strains belonging to the species, *Hanseniaspora uvarum*, *Kloeckera apiculata*, *Metschnikowia pulcherrima,* and *P. anomala,* can be responsible for ester taint in wines, especially if their growth is not controlled during the initial stages of alcoholic fermentation (Loureiro and Malfeito-Ferreira, 2003). The compounds formed are the esthers ethyl acetate, with an acetone/glue aroma, and methylbutyl acetate, that presents as banana or pear drops with a fruity aroma.

The presence of too much ethyl acetate in wine is not wanted due to its unpleasant acetone/glue aroma, especially if it is present in concentrations above 100–200 mg/L (Cliff and Pickering, 2006). Ethyl acetate is formed in wine through the action of yeast or via esterification. The final concentration is dependent on the species of yeast and the wine composition (Plata et al., 2003).

Carbonic maceration, must aeration, must clarification, and yeast species play an important role in the amounts of isoamyl acetate formed in wines. This compound possesses a banana-like odor and may also impart some complexity to neutral white wines and to red and white premier wines. When present in excess it can cover the varietal character of wines (Ribéreau-Gayon et al., 2006a).

Growth of *Z. bailii* may also lead to wine with an increase in acetic and succinic acid, a decrease of L-malic acid, reduction in total acidity, and an altered ester concentration (Boulton et al., 1996).

2.3 "Brett Character" and Mousy Taint: Unpleasant Horsy Aroma and Mice Taste in Wines

Some wine microorganisms can produce volatile phenols (vinylphenols and ethylphenols) from grape *p*-coumaric and ferulic acids. *Brettanomyces bruxellensis* produce above-threshold concentrations of "phenolic," "animal," or "stable" odor-active ethylphenols like 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG), by decarboxylation of the *trans*-ferulic and *trans-p*-coumaric precursor compounds present in wine (Chatonnet et al., 1997).

Dekkera and its anamorph *Brettanomyces* are considered spoilage yeast and are probably the most important organisms responsible for the production of volatile phenols (Chatonnet et al., 1992). They are not among the most dominant microorganisms during fermentation; however, in wines and in other fermented beverages, such as beer and cider, they can be found after 6–10 months of storage (Cocolin et al., 2004). Optimal substrates for the proliferation of *Brettanomyces* are wood barrels. Wine maturation and storage in wood, with lower levels of sulfur dioxide, some dissolved oxygen, and storage at higher temperature than at any other time, provides both the time and the lack of competition needed for *Brettanomyces* to successfully grow to levels that result in wine sensory defects (Lin et al., 2014). For all these reasons, it is thought that the time between the completion of alcoholic fermentation and the start of MLF is the most likely time when *Brettanomyces* multiplies and produces "Brett character" in wine (Lin et al., 2014). Furthermore, it is thought that these yeasts can also multiply after bottling if the wine contains residual fermentable sugars, a situation made more likely if the wine was minimally filtered (Du Toit and Pretorius, 2000).

Nevertheless, *Brettanomyces* occurs almost exclusively in red wines. Red wines have a much higher level of phenolic acids than white wines, since they are extracted from the grape skins during red wine fermentation. The mechanism of conversion involves a sequence of two enzymatic reactions. The wine yeast *S. cerevisiae* and some LAB, such as *Lactobacillus* have enzymes (decarboxilases), which degrade these acids to weakly smelling intermediates like 4-vinylphenol and 4-vinylguaiacol. These compounds, by the enzimatic mechanism of *Brettanomyces*, are transformed to the off-odors 4-ethylphenol and 4-ethylguaiacol, respectively (Chatonnet et al., 1995). In wine, *Brettanomyces* is the only yeast that can

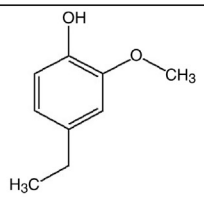
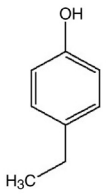
Name	Abbreviation	Structure	MW	log *P*	Perception threshold (μM)
4-Ethylguaiacol	4-EG		152.19	2.38	0.92
4-Ethylphenol	4-EP		122.16	2.55	5.08

Figure 9.7: Structures and Chemical Properties of 4-Ethylguaiacol (4-EG) and 4-Ethylphenol (4-EP).

produce from 4-vinylphenol the horsy smelling 4-ethylphenol. Usually, 4-ethylphenol is considered to impart the characteristic "Brett character" aroma profile of *Brettanomyces* wine contamination (Couto et al., 2005) (Figs. 9.7 and 9.8).

The perception threshold of EPs (4-ethylphenols, designated as 4-ethylphenol and 4-ethylguaiacol) is greatly influenced by the matrix in which they are dissolved. The values reported by Chatonnet et al. (1992)—440 μg/L for 4-EP and 135 μg/L for 4-EG—were found in a model solution. In red wines the 4-ethylphenol presents a detection threshold of 230 μg/L (Chatonnet et al., 1990) while the combination of 4-ethylphenol with 4-ethylguaiacol shows a threshold of 400 μg/L (Chatonnet et al., 1992) and this happens when active *Brettanomyces* in the wine overcome 10^3 cells/mL (Chatonnet et al., 1995; Lonvaud-Funel and Renouf, 2005). The concentration of 4-EP found in 61 bottled commercial Australian red wines, of various ages, ranging from 2 μg/L in a Merlot up to 2660 μg/L in a Shiraz, with a mean concentration of 795 μg/L. 4-EG was also detected in every red wine analyzed, ranging in concentration from 1 μg/L (in a Pinot noir) up to 437 μg/L (in a Merlot) with a mean concentration of 99 μg/L (Pollnitz et al., 2000).

However, the presence of 4-vinylphenol and 4-ethylguaiacol, in wine, can be due to the metabolism of *Saccharomyces* yeast during grape-must fermentation (Gunata et al., 1990). Meaden and Taylor (1991) cloned and identified a nuclear gene phenolic off-flavor (POF 1) that confers, to the yeast *S. cerevisiae*, the ability to carry out the decarboxylation of *p*-coumaric and ferulic acid. Grando et al. (1993) verified the character expressed by strains Pof^+ and Pof^- in fermentation of Traminer grape-musts, confirming the influence of the yeast strain Pof phenotype from analytical results of 4-vinylphenol and 4-ethylguaiacol and from sensorial evaluation. Some of the genetic and environmental factors that affect Pof phenotype on *S. cerevisiae* are today well understood (Howell et al., 2005).

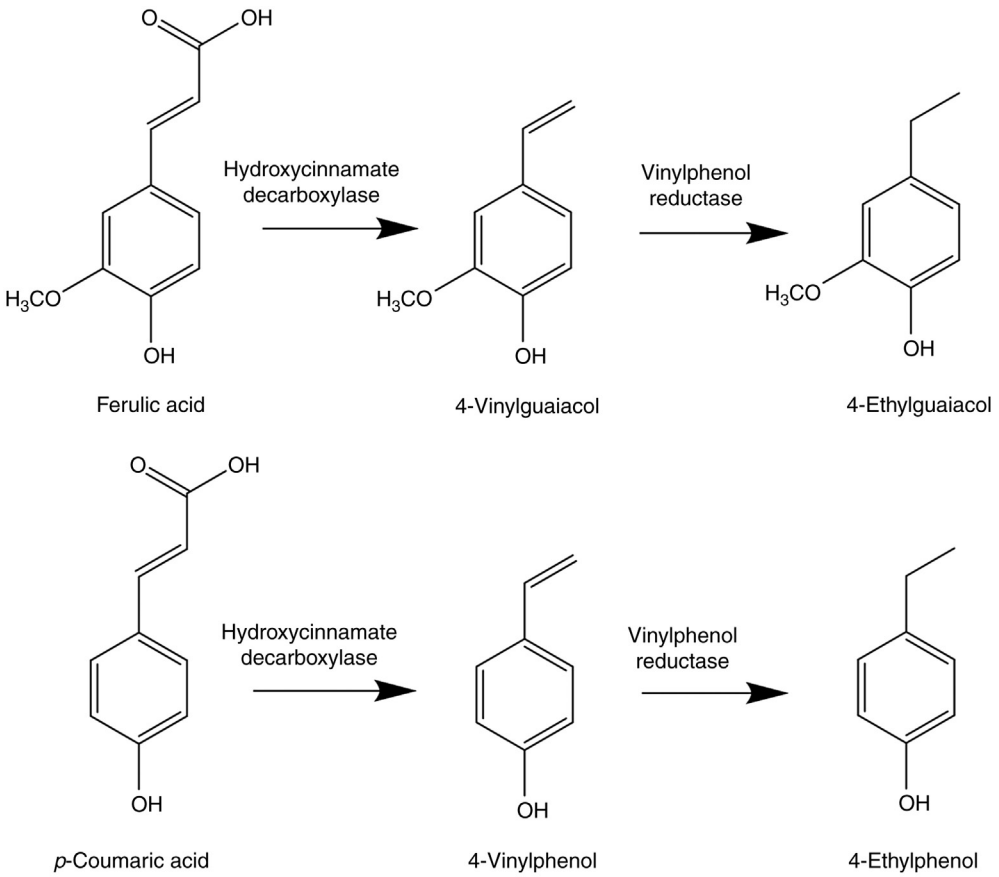


Figure 9.8: Formation of Ethylphenols from Hydroxycinnamic Precursors.

Under laboratory conditions, volatile phenols can also be produced by LAB (Cavin et al., 1993; Couto et al., 2006). Cavin et al. (1993) found that strains of the genera *Lactobacillus* and *Pediococcus* can produce volatile phenols. Couto et al. (2006) after testing 20 different species of LAB (35 strains) found that only *L. plantarum*–type strain NCFB1752 produced the predictable quantity of 4-vinylphenol and 4-ethylphenol from *p*-coumaric acid. However, the production of these off-flavors by *L. plantarum* NCFB1752 was affected by the growth conditions/environmental factors (Silva et al., 2011a, 2011b) and yielded lower concentration of these compounds than those normally obtained by *Dekkera/Brettanomyces* yeasts, considered to be the principal agents responsible for volatile phenols in wine (Fras et al., 2014).

Besides "Brett character" *Brettanomyces* also can produce mousy wines. This wine defect appears due to the metabolism of ornithine and lysine that leads to the production of extremely powerful and unpleasant nitrogen-heterocylic compounds [2-acetyltetrahydropyridine

(ACTPY), 2-acetyl-1-pyrroline (ACPY), and 2-ethyltetrahydropyridine (ETPY)] (Costello and Henschke, 2002). These molecules are recognized on the back palate (retro-nasal area) as a persistent aftertaste suggestive of caged mice (Grbin et al. 1996). The temperature of the mouth (near 36°C) associated with the pH of basic saliva neutralizes the wine's acid pH. This unpleasent flavor is perceived in the mouth and is often called "mice taste" (Grbin and Henschke, 2000).

The minimum population of yeast required for producing "mice taste wines" or "horsy wines" may account for the differences in the incidence of these two *Dekkera/Brettanomyces* spoilage phenomena. Chatonnet et al. (1995) determined that a population of 3×10^3 cells/mL was sufficient to produce an above-threshold concentration of 4-ethylphenol in a red wine, while based on the data presented by Grbin and Henschke (2000), a higher population could be necessary for mousy off-flavor development.

2.4 Formation of Ethyl Carbamate and Biogenic Amines

The amino acid metabolism by microorganisms can have implications on quality and safety of fermented food products. For bacteria, the amino acid use can increase their ability to obtain energy, particularly in media with nutrient limitation and in response to acid stress (Cotter and Hill, 2003).

Arginine is one of the amino acids present in higher concentrations, in must and wine. The wine lactic acid bacteria degrade arginine by arginine deaminase pathway that involves three enzymes: arginine deaminase (ADI, EC 3.5.3.6), ornithine transcarbamylase (OTC, EC 2.1.3.3), and carbamate kinase (CK, EC 2.7.2.2) (Lasik, 2013; Liu, 2002; Manca de Nadra et al., 2003; Mira de Orduna et al., 2001). Its degradation yields citrulline and carbamoyl phosphate, precursors of ethyl carbamate, a potentially carcinogenic compound. This compound, also referred to as urethane, results from a spontaneous chemical reaction involving ethanol and precursors including urea, citrulline, carbamoyl phosphate, *N*-carbamyl α- and β-aminoacids and allantoin (Ough et al., 1988a). The ethanolysis reaction of citrulline and urea (from the metabolism of arginine by yeasts) (Vincenzini et al., 2009) to form ethyl carbamate can occur at elevated temperatures or at normal storage temperatures (Ough et al., 1988a). The presence of the three enzymes of the ADI pathway appears to occur in most heterofermentative genera, *Lactobacillus*, *Leuconostoc*, and *Oenococcus*, although already detected in some homofermentative LAB species isolated from wine. However, the degradation of arginine by this pathway, in all species, seems to be a strain-dependent phenotype (Spano et al., 2006). The genes involved in the degradation of arginine are arranged in *arc* operon. While this operon structure presents variations between different species of LAB, the genes coding for arginine deaminase (*arc*A); ornithine transcarbamylase (*arc*B), and carbamate kinase (*arc*C) are always present (Divol et al., 2003). This operon has been described in *Lactobacillus hilgardii* (Arena et al., 2002, 2013), *O. oeni* (Inês, 2007; Mira de Orduna et al., 2001; Nehmé

et al., 2006), *L. plantarum* (Spano et al., 2006), and *L. sake* (Zuniga et al., 2002). In wine, prolonged contact of LAB (viable and viable but not culturable) with the yeast lees should be considered as a significant risk factor for the increased formation of citrulline and thus ethyl carbamate (Terrade and Mira de Orduna, 2006; Tonon and Lonvaud-Funel, 2000). So, it is not advisable to use strains of *O. oeni* that excrete citrulline as starter cultures. Some of these authors also suggest that strains having only the first pathway enzyme (ADI+, OTC–) or strains that have ADI but low OTC activity should be excluded in the process of selection of starters for the realization of MLF. According to Vincenzini et al. (2009), urea is considered the major precursor for urethane in wine and it can be released by wine yeasts as a metabolic intermediate from arginine breakdown by arginase enzyme. Urea excretion principally occurs during the first stages of fermentation and is potentiated by conditions of high availability of quickly assimilable nitrogen sources.

The BA are organic low molecular weight bases, which can be produced and degraded during normal metabolic activity of animals, plants, and microorganisms (Arena and Manca de Nadra, 2001). In humans, these substances may play an important metabolic role related to growth (polyamines) or functions of the nervous and circulatory systems (histamine and tyramine). When BA are ingested in excess they can cause hypotension, hypertension, heart palpitations (vasoactive amines), headaches (psychoactive amines), and various allergic reactions (De las Rivas et al., 2005; Kushnereva, 2015; Lonvaud-Funel, 2001). The BA are essentially formed from the decarboxylation of their respective precursor amino acids (Guerrini et al., 2002; Lasik, 2013; Lonvaud-Funel, 1999; Marcobal et al., 2006a,b; Vincenzini et al., 2009). Thus, amines histamine, tyramine, tryptamine, serotonin, 2-phenylethylamine, agmatine, and cadaverine are formed from the amino acids histidine, tyrosine, tryptophan, hydroxytryptophan, phenylalanine, arginine, and lysine, respectively (Buckenhüskes, 1993; Henríquez-Aedo et al., 2016; Kalac and Krizec, 2003). The aliphatic volatile amines (methylamine, ethylamine, and isoamylamine) can be originated by the amination of nonnitrogen compounds, such as aldehydes and ketones. The putrescine may be formed from agmatine or ornithine and the polyamines spermidine and spermine are formed from putrescine by binding aminopropyl groups catalyzed by spermidine synthase and spermine synthase (Vincenzini et al., 2009).

The formation of biogenic amines (BA) by BAL may occur during the fermentation processes of various raw materials for obtaining foods and beverages, such as cheese, sausages, fermented vegetables, beer, and wine. Many bacteria have decarboxylase activities, which promote growth and survival in acidic environments, by a pH increase. In wine, various amino acids can be decarboxylated and therefore BA may be found, predominating histamine, tyramine, putrescine, the isopentylamine, and phenylethylamine (Lonvaud-Funel, 2001; Moreno-Arribas et al., 2003). Although some BA can be derived directly from grapes and others result from the metabolic activity of the *Saccharomyces* and non-*Saccharomyces* yeast and acetic bacteria, BA normally increase after MLF (Landete et al., 2005; Lonvaud-Funel,

2001; Pramateftaki et al., 2006; Torrea-Goni and Ancín-Azpilicueta, 2001). Results for yeast contribution in the BA production are very contradictory. Caruso et al. (2002) found that different species, such as *K. apiculata*, *M. pulcherrima*, *B. bruxellensis,* and *S. cerevisiae* produced *2*-phenethylamine and agmatine; however, Landete et al. (2007) stated that none of the 36 yeast strains (belonging to 12 different species) evaluated for BA production were able to produce BA (histamine, tyramine, 2-phenethylamine, putrescine, cadaverine, and tryptamine) in synthetic medium and grape must. Among the LAB, the decarboxylase activity is strain-specific and within different species of *Lactobacillus*, *Pediococcus*, *Leuconostoc,* and *Oenococcus* is randomly distributed. In wine, however, their levels are much lower than those described for other foods, though ethanol can enhance the toxic effect of histamine, by inhibiting amino oxidases. Currently, most of the EU countries and Canada recommend histamine levels not exceeding 10 mg/L, while in Germany the limit is 2 mg/L. Besides their toxicity, at higher concentrations, BA may confer detectable unpleasant sensory changes, such as putrescine and cadaverine, with rotten fruit and rotten flesh aromas, respectively. The appearance of BA in wine has been linked to the existence of precursor amino acids, strains with descarboxilase activity of factors that affect the growth of these strains, as well as some enological practices (González-Marco and Ancín-Azpilicueta, 2006; Lonvaud-Funel, 2001; Martín-Álvarez et al., 2006), such as nutrient addition, prolonged contact with grape skins and lees (Lonvaud-Funel, 2001; Vincenzini et al., 2009) during fermentation process, because they can directly affect the content of the precursor amino acids of BA (Vincenzini et al., 2009). Red wines generally have higher contents of BA compared to pink, white, and fortified wines (Bover-Cid et al., 2006; Landete et al., 2005; Leitão et al., 2000).

2.5 Oxidized Taint From Acetaldehyde: Grass, Green Apple, Sherry Taint

For human health, acetaldehyde causes some concern since it is also implicated in hangovers and severe headaches. In wine it can impart some undesirable flavors, when above a certain concentration. Acetaldehyde is an intermediate product of yeast fermentation; however, it is more commonly associated with ethanol oxidation, catalyzed by the enzyme ethanol dehydrogenase. Acetaldehyde production is also associated with the presence of surface-film-forming yeasts *Candida* sp., *Pichia* sp., and *Hansenula* sp., *S. ludwigii*, and bacteria, such as acetic acid bacteria, which form the compound by the decarboxylation of pyruvate. The sensory threshold for acetaldehyde is 100–125 mg/L (Carlton et al., 2007). Beyond this level, it imparts a sherry-type character to the wine that can also be described as green/baked apple and very ripe persimmon. Only in sherry wines, the oxidation character of acetaldehyde is considered a typicity of the wine, and not a wine fault. In this type of wines the acetaldehyde concentration may reach to values closer to 500 mg/L (Zoecklein et al., 1995).

However, wine aroma is influenced by complex interactions between various wine constituents. Recently, Coetzee et al. (2016) investigated the sensory interactive effects of Sauvignon Blanc impact compounds, 3-mercaptohexan-1-ol and 3-isobutyl-2-methoxypyrazine, Sauvignon

Blanc sensory markers, with acetaldehyde. They found that complex sensory interactions may occur between these compounds and one of the main oxidation-derived compounds, acetaldehyde. Acetaldehyde can enhance or suppress pleasant fruity characters depending on the concentration.

2.6 Production of Mycotoxins

Mycotoxins are small molecules (MW~700), natural grape contaminants, that are very toxic chemical products produced as secondary metabolits by a few fungal species. The most important include AFs, fumonisins (FB1 and FB1), and ochratoxin A (OTA) (García-Cela et al., 2012; Hathout and Aly, 2014). The conditions necessary for the production of mycotoxins by fungal, depends on availability of nutrients, moisture, pH, temperature, and strain (Fung and Clark, 2004). The most dangerous mycotoxins are the AFs, which are mainly produced by *A. flavus* and *A. parasiticus* (Hathout and Aly, 2014). The chemical structure of AFs consists in a difuranocoumarin derivate produced by a polyketide pathway (García-Cela et al., 2012). Ochratoxins are produced by filamentous fungal species belonging to the genera *Penicillium* and *Aspergillus* (Caronel et al., 2011; Gil-Serna et al., 2011). This mycotoxin is a colorless crystalline compound. Its chemical structure consists of a dihydroisocoumarin moiety linked to L-β-phenylalanine by a peptide bond (García-Cela et al., 2012; Quintela et al., 2012a, 2012b). Fumonisines were mainly produced by different strains of *Fusarium* (Friavad et al., 2007), with fumonisin B1 being the most important and chemically the diester of propane-1,2,3-tricarboxylic acid and 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyeicosane (Lino et al., 2004).

3 Current Methods for the Reduction of Volatile Acidity in Wines and Grape Musts

Several methodologies, aiming to decrease excessive VA of acidic wines, have been proposed (Vilela-Moura et al., 2011): (1) microbial stabilization of the acidic wine followed by mixture with other wines, (2) reverse osmosis (RO) and nanofiltration, and (3) biological removal of acetic acid through refermentation (Ribéreau-Gayon et al., 2006b). In the first approach the acidic wine is blended with other wines with low acetic acid. The acidic wine can be sold for distillation to the ethanol industry, with economical losses for the producers. RO and nanofiltration, two new membrane filtration techniques, can also be used for the deacidification of wines. These methods produce an acetic-acid-rich permeate, which is then treated by ion exchange to remove the acetic acid (Boulton et al., 1996). Several corporations market RO systems for wine VA reduction. A Californian company (Vinovation) proposed coupling RO and anion exchange resins (U.S. Patent No. 08/218,920), whereas, in a second approach, the company "VA Filtration" proposes a combination of RO and selective adsorption of acetic acid, with a reduction of 30% in VA on a single pass (VA Filtration,

2015). According to Massot et al. (2008), a third approach consists of the combination of two stages of RO, where the targeted acid of the first permeate is transferred into a salty form and then retained by the second stage RO membrane.

3.1 Biological Deacetification of Musts and Wines

In order to achieve wines with balanced sugar and acid contents, several approaches have been developed for biodeacidification; however, they are limited to the metabolism of malic acid (Bony et al., 1997; Husnik et al., 2007). Concerning VA, a genetically modified strain that decreases acetate has been obtained (Remize et al., 2000). However, such a strain will not be used for winemaking in the near future, due to the controversy regarding the use of genetically modified foods in Europe (Schuller, 2010). Abnormally high concentrations of acetic acid in wines can be removed by refermentation. Yeasts are able to metabolize acetic acid during a refermentation process (Ribéreau-Gayon et al., 2000a). Acording to these authors and the works by Vilela-Moura et al. (2008, 2010, and 2011), one third of acidic wine is mixed with two thirds of freshly crushed grapes or of the residual marc from the fermentation of a finished wine (remaining pulp, after draining the newly made wine), such that the VA of this mixture does not exceed 0.73 g/L of acetic acid. This empirical methodology reduces VA to values closer to 0.37 g/L of acetic acid and has relatively low costs (Zoecklein et al., 1995).

3.1.1 Acetic acid catabolism in yeast

As referred in the introduction, acetic acid in grape-must or wines can be the product of fungi, bacteria, or yeast metabolism. The ability of yeasts to catabolize acetic acid can be especially exploited to develop methods for the zymological deacidification of grape-musts or wines.

Acetate can be used as a sole carbon and energy source for the generation of energy and cellular biomass under aerobic conditions (Schüller, 2003). In *S. cerevisiae*, acetate transport and metabolism are subject to glucose repression. Therefore, when grown in medium containing glucose and acetic acid, *S. cerevisiae* displays a diauxic growth with consumption of acetic acid only after glucose reduction (Rodrigues, 1998). However, in chemostat cultures of *S. cerevisiae* grown in mixtures of glucose and acetic acid, the glucose concentration can be so low that the cells are no longer repressed and can metabolize acetate concomitantly with glucose (dos Santos et al., 2003). This behavior is identical of the species *Z. bailii*, which displays a biphasic growth in medium containing glucose and acetic acid (Sousa et al., 1998).

A review made by Vilela-Moura et al. (2011) discusses the catabolism of acetic acid in yeast, including its cellular uptake/transport, which is important to stimulate its degradation reducing acetic acid concentration in grape-musts and wines. In glucose-repressed yeast cells at low pH, where acetic acid is mostly undissociated (pKa 4.75), it enters mainly by simple diffusion (Casal et al., 1996). Ethanol enhances the passive influx acetic acid, which

follows first-order kinetics with a rate constant that increases exponentially with ethanol concentration (Casal et al., 1998). It is known that Jen1p is required for the uptake of lactate in *S. cerevisiae* and can also transport other monocarboxylates, including acetate (Casal et al., 1999). The protein Ady2p was later found to be essential for acetate transport activity in acetic acid-grown cells (Paiva et al., 2004). When available as the sole carbon and energy source, acetate is metabolized to acetyl coenzyme A (acetyl-CoA) by one of the two ACS proteins: Acs1p (peroxisomal) or Acs2p (cytosolic). Acetyl-CoA is then oxidized in the tricarboxylic acid cycle after entering mitochondria. It is also used to produce succinate replenishing the cell with biosynthetic precursors by entering the glyoxylate cycle (Fig. 9.9), which involves the key enzymes isocitrate lyase (Icl1p) and malate synthase (Mls1p) in the cytosol and peroxisome, respectively (Vilela-Moura et al., 2011). In addition, acetyl-CoA is used for synthesis of macromolecules, which requires active gluconeogenesis (Kruckeber and Dickinson, 2004).

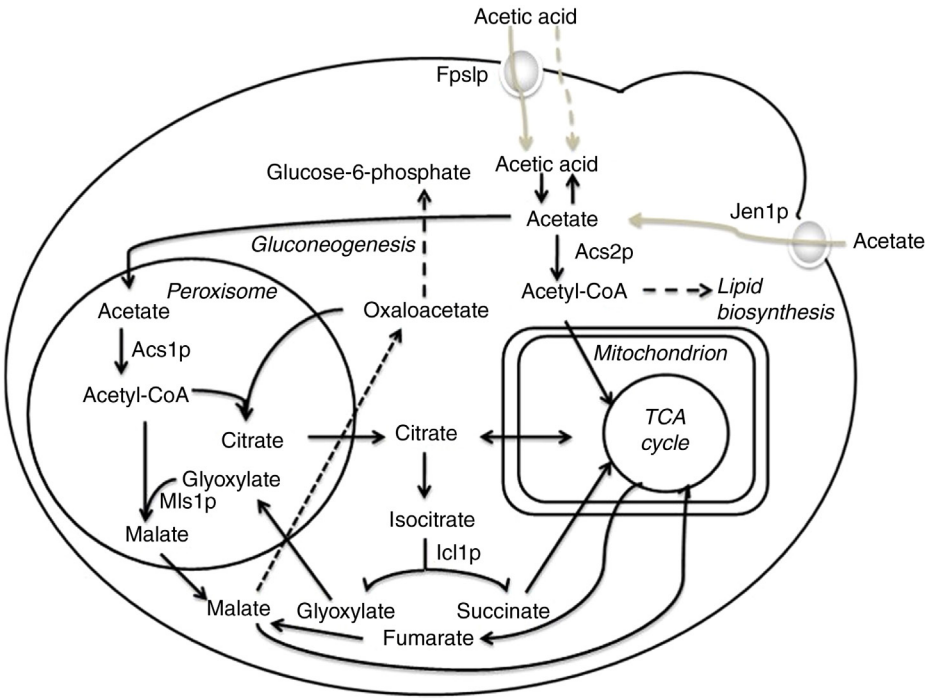


Figure 9.9: Cell Compartmentation of Acetate Metabolism in Yeast.
When available as the sole carbon and energy source, acetate is metabolized to acetyl-CoA by one of the two ACS proteins: Acs1p (peroxisomal) or Acs2p (cytosolic). Acetyl-CoA is then oxidized in the tricarboxylic acid cycle after entering mitochondria. It is also used to produce succinate and hence replenish the cell with biosynthetic precursors by entering the glyoxylate cycle, which involves the key enzymes isocitrate lyase (Icl1p) and malate synthase (Mls1p) in the cytosol and peroxisome, respectively. In addition, acetyl-CoA is used for synthesis of macromolecules, which requires active gluconeogenesis (Vilela-Moura, 2010).

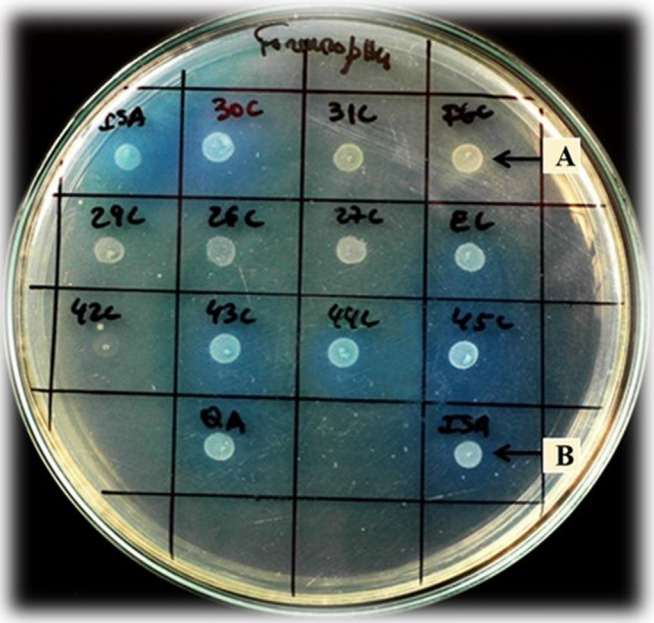


Figure 9.10: Growth and Color Change (due to pH Changes) of the Differential Medium, With 0.5% (v/v) Acetic Acid, 0.05% (w/v) Glucose, and Bromocresol Green 0.005% (w/v), at pH 4.0, Indicating Simultaneous Consumption of Glucose and Acetic Acid by the Isolated Strains 30, 43, 44, and 45C.

(A) *S. cerevisiae* PYCC 4072 (negative control); (B) *Z. bailii* ISA1307 (positive control). *Adapted from Schuller, D., Côrte-Real, M., Leão, C., 2000. A differential medium for the enumeration of the spoilage yeast* Zygosaccharomyces bailii *in wine. J. Food Prot. 63 (11), 1570–1575; Vilela, A., Amaral, C., Shuller, D., Mendes-Faia, A., Corte-Real, M., 2015. Combined use of Wallerstein and* Zygosaccharomyces bailii *modified differential media to isolate yeasts for the controlled reduction of volatile acidity of grape musts and wines. J. Biotechnol. Res. 6, 43–53.*

3.1.2 Volatile acidity bioreduction by Saccharomyces cerevisiae yeast strains

Vilela et al. (2015) isolated several yeast strains (*Saccharomyces* and non-*Saccharomyces*) in Wallerstein Laboratory Nutrient Agar (WL) medium from refermentation processes of acidic wines, at winery scale. Among these isolates, 135 were tested for their ability to consume acetic acid in the presence of glucose, using a differential medium adapted of the one created by Schuller et al. (2000) containing acetic acid and glucose. Four isolates (Fig. 9.10) were obtained in this medium and were characterized by molecular methods that confirmed the presence of a non-*Saccharomyces* strain (*L. thermotolarans* coded as 44C) and three isolates of *S. cerevisiae* (30, 43, and 45C) (Vilela et al., 2015).

Vilela-Moura et al. (2008) also found that consumption of glucose and acetic acid was, likewise, an attribute of commercial wine yeasts. They characterized nine *S. cerevisiae*

commercial strains regarding this particular physiological feature. Among the strains studied, S26, S29, and S30 displayed a simultaneous consumption pattern of glucose and acetic acid. Afterward, these strains were further assessed in simulated refermentation assays of acidic wines with a synthetic culture medium containing high glucose and low ethanol concentrations, simulating the process of refermentation with a grape-must; and low glucose and high ethanol concentrations, simulating the process of refermentation with a marc. Commercial strain S29 was the most efficient one in the simulated refermentation assay with must, and under low oxygen availability. Conversely, for the simulated refermentation assay with marc, under aerobic conditions, S26 was the most efficient acetic acid-degrading strain. Under limited-aerobic conditions all the *S. cerevisiae* strains evaluated displayed acetic acid degradation efficiencies identical to *Z. bailii* ISA 1307. Remarkably, S26 strain also revealed capacity to decrease 92% and 61%, the VA of wines, without the addition of grape-must or marcs, in aerobic and limited aerobic conditions, respectively (Vilela-Moura et al., 2008).

3.1.3 Effects of ethanol, acetic acid, sulfur dioxide, and microoxygenation (MO) on the removal of volatile acidity from acidic wines by Saccharomyces cerevisiae strains

Upon inoculation of grape-must, yeast cells need to adapt to a fermentative environment that gradually changes during fermentation and that imposes multiple stress conditions, such as high osmolarity, low pH (2.9–3.8; Pizarro et al., 2007), sulfur dioxide (SO_2) presence between 40 and 100 mg/L (Ribéreau-Gayon et al., 2000a), ethanol toxicity (Ribéreau-Gayon et al., 2000a), temperature variations (Pizarro et al., 2007), and increasing nitrogen limitation (Martínez-Moreno et al., 2012). A refermentation process that aims to reduce excessive VA imposes additional stress due to elevated acetic acid concentrations that may lead to a reduced cellular growth, cellular death, and, consequently, to stuck fermentations (Eglinton and Henschke, 1999). A controlled oxygen environment is also important for yeast acetic acid consumption (Vilela-Moura et al., 2008).

Controlled wine oxygenation is currently achieved through microoxygenation (MO). By this technique small amounts of oxygen are delivered along fermentation. Oxygen is usually added by a stainless steel sparger that produces small bubbles, promoting the dissolution of oxygen. The aim of MO is to provide oxygen at a rate equal to or slightly less than the wine's oxygen consumption rate, to avoid the buildup of too much oxygen in the wine (Llaudy et al., 2006; Tao et al., 2007). This procedure has an impact on multiple aspects of wine production, such as increased production of sterols and other fatty acids by yeast (Zoecklein et al., 1995), removal of unwanted reductive flavors (Paul, 2002), and reduced vegetative aromas (McCord, 2003).

The influence of different combinations of the initial concentration of acetic acid, ethanol, sulfur dioxide, and MO on acetic acid removal from acidic wines by two commercial *S. cerevisiae* strains S26 and S29 was evaluated by Vilela-Moura et al. (2010a,b). Both strains reduced the VA of an acidic wine [1.0 g/L acetic acid, 11% (v/v) ethanol] by 78 and 48%,

respectively. Strain S26 revealed better removal efficiency due to its higher tolerance to stress factors imposed by acidic wines. Sulfur dioxide (SO_2) in the concentration range 95–170 mg/L inhibited the ability of both strains to reduce the VA of the acidic wine under the experimental conditions used. Deacidification of wines with the better performing strain was associated with changes in the concentration of volatile compounds. Moreover, deacidification led to an increased on fatty acids concentration, but still within the range of values described for spontaneous fermentations, and with apparently no negative impact on the organoleptical properties (Vilela-Moura et al., 2010a).

Afterward, the applicability of the aforementioned characterized commercial and indigenous *S. cerevisiae* strains for the deacidification of white and red wines at a pilot scale was determined (Vilela-Moura et al., 2010b). The effect of the refermentation process, as well as MO (20 mg/Lh of oxygen applied with a MicroSafeO_2) on acetic acid removal efficiency and wine aromatic composition was assessed in a red wine. The commercial strains S26 and S29 efficiently reduced both acetic acid (43 and 47%, respectively) and sugar (100%) after 264 h of refermentation of an acidic white wine that was supplemented with grape must. Similar results (60%–66% of acetic acid removal) were observed for red wine deacidification using grape must, independently of MO. Wines obtained by refermentation with the must had significantly lower acetic acid and a higher total SO_2 concentration in comparison to the wines deacidified by the grape marcs. The volatile aroma compound's composition of deacidified red wines was dependent on the refermentation process used, rather than on MO. The marc-deacidified wine obtained by the use of strain S26 and without MO achieved the best sensory classification. When data from all analytical and sensory evaluation were combined, principal component analysis separated the wines into three distinct groups according to the strain and the refermentation process independently of MO (Vilela-Moura et al., 2010b).

3.1.4 Bioreduction of volatile acidity by immobilized Saccharomyces cerevisiae cells

Cell immobilization by entrapment in beads is a technique that received increasing attention in recent years, resulting in a great number of applications in industry (Yamada et al., 2002), medicine, and agriculture (Liu et al., 2007). Compared with suspension cultures, this technique offers the advantages of continuous cell utilization in addition to the protection of immobilized cells against inhibitory substances in the fermentation medium, increased fermentation rates, stimulation of production, and protection from shear forces (Riley et al., 1990).

Vilela et al. (2013) studied the efficiency of acetic acid removal from acidic wines by immobilized cells of the previously characterized commercial strain S26. The effect of different parameters for cellular immobilization, such as cell concentration, initial pH, and number/composition of the immobilization matrix on the deacidification process efficiency was also determined. Immobilized cells using double layer alginate-chitosan beads were

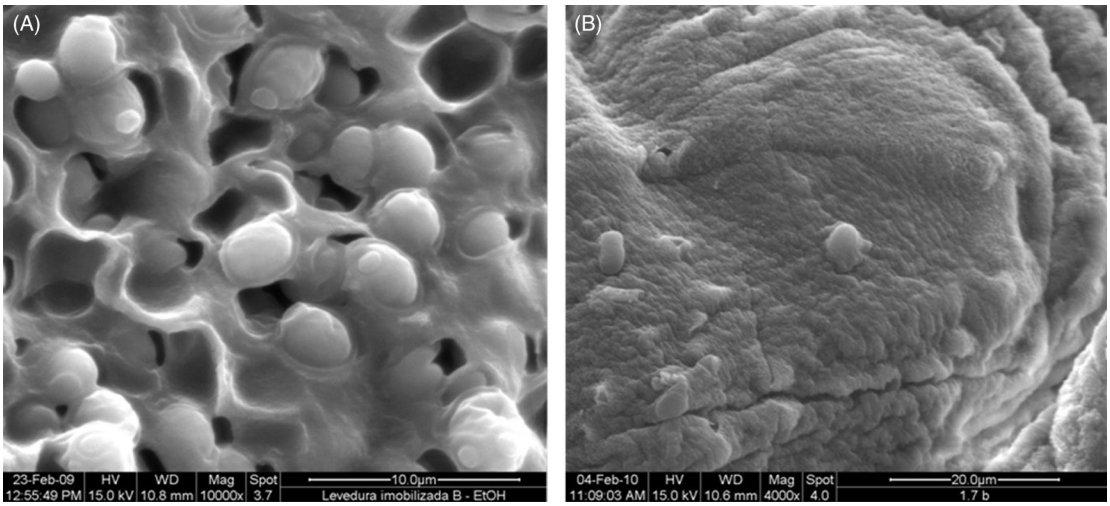


Figure 9.11: SEM Image of *S. Cerevisiae* S26 Cells, Entrapped in Double Layer (DL) Alginate-Chitosan Beads.

(A) Internal yeast cells distribution of a double-layer bead with alginate 2.0% (w/v) and chitosan 1.0% (w/v) after deacidification at pH 3.12 (10,000×); (B) External layer (chitosan 1.0%, w/v) of a bead after deacidification, pH 3.12 (5,000×) (Vilela et al., 2013).

able to reduce 21.6% of the initial VA of an acidic wine (1.1 g/L acetic acid) with 12.5% (v/v) ethanol and pH 3.5, after 72 h. Deacidification did not change after 168 h and was associated with a slight ethanol decrease and cell leakage from the beads. Duplication of initial cell concentration and pH adjustment to 3.12 lead to an increase of VA removal up to 61.8%, depending on the initial acetic acid concentration. No cell leakage occurred during the process, whereas ethanol decreased slightly (0.7%, v/v). SEM analysis of immobilized cells confirmed these results, suggesting that the initial pH value is critical for beads integrity maintenance (Fig. 9.11). Thus, immobilized S26 cells in double layer alginate-chitosan beads appear to be an efficient alternative to improve wine quality by correction of excessive VA. In summary, the results mentioned earlier, are the basis to develop efficient and cheap enological solutions for the rectification of VA of wines using free or immobilized select yeast strains.

4 Role of LAB on Wine Safety and Quality

LAB are important since they perform the MLF reducing the wine acidity, important in red wines contributing to microbiological stability and sensory quality of the final product (Maicas, 2001; Sumby et al., 2014). Phenolic compounds, mainly phenolic acids and their esters and some flavonols, stimulate bacterial growth (Campos et al., 2003) and are metabolized during MLF, but LAB also increase the wine with new phenolic compounds not detected in the initial wine (Hernández et al., 2007). Consequently, it may be suggested that LAB could be among the factors that contribute to the antioxidant activity of wine. There are also LAB species with potential to hydrolyze tannins. Tannin acyl hydrolase (E.C. 3.1.1.20),

commonly called tannase, catalyzes the hydrolysis of ester bonds in hydrolyzable tannins, such as tannic acid, thereby releasing glucose and gallic acid (Aguilar and Gutierrez-Sanchez, 2001). This enzyme also hydrolyzes the esters bonds from polyphenols, avoiding their polymerization, giving a wine with a high content of aromatic compounds and appropriate color, increasing its quality. The tannase activity could be considered an important criterion for the selection of malolactic starter cultures (Buckenhüskes, 1993) since it may confer advantages in the winemaking process by reducing astringency and haze in wine (Vaqueres Vaquero et al., 2004).

Is well known that LAB are considered health beneficial microorganisms because they are traditionally used in the production of fermented food and feed products, and because they have several well-known probiotic properties, thus being considered as GRAS (generally recognized as safe) microorganisms. Lately, they also began to be recognized for their ability to detoxify mycotoxins (Dalié et al., 2010; Salminen et al., 2010), thus being considered a promising solution to reduce exposure to dietary mycotoxins. This unusual LAB characteristic is strain specific and involves adsorption and metabolization mechanisms. Ochratoxin A (OTA) is one of the most prominent mycotoxins found in agricultural commodities, including wine. It was reported by Abrunhosa et al. (2014) that the ability of *Pediococcus parvulus* strains isolated from Douro wines that spontaneously underwent MLF to detoxify OTA. These strains have a potential value that can be exploited for applications in food and feed, as probiotic for animal nutrition, as inoculants for silages, as biocontrol agents in crop fields, as starter cultures for fermented products, and/or as starter cultures for wine MLFs.

Beside OTA removal or degradation, also BA degradation is another important role of LAB in the destruction of metabolites of microbial origin with an impact on consumer health (Russo et al., 2016). BA in wine represent a toxicological risk for the health of the consumer, with several trade implications. Capozzi et al. (2012) isolated wine *L. plantarum* strains able to degrade BA, thus with added potential for being used as malolactic starter cultures. In a recent study, Callejón et al. (2014) verified a high degradative power of histamine, tyramine, and putrescine by wine LAB strains belonging to *L. plantarum* and *P. acidilactici* species.

5 *Preventive Treatments to Avoid* Brettanomyces *sp. Wine Contamination*

5.1 *Sulfur Dioxide*

The most commonly used additive to control the growth of microorganisms in wine is sulfur dioxide (SO_2). However, starting from the late 1970s, the use of sulfite in food has been questioned because of its allergenicity identified by the European Food Safety Authority (EFSA, 2004). EFSA, leading the European Union (EU), the USA, Australia, and other countries to implement labeling frameworks for foods containing more than 10 mg/L of this additive (European Union, 2011). The addition of this compound is legal and effective, and it

can inhibit the growth of *Dekkera/Brettanomyces* when the concentration of free SO_2 is just under 20 mg/L at pH 3.6–3.7 (Chatonnet et al., 1993).

Portugal et al. (2014) compared several antimicrobial agents, such as dimethyl dicarbonate (DMDC), chitosan, potassium metabisulphite (PMB), and enological tannins against *Brettanomyces*, and they verify that PMB was the most efficient antimicrobial agent in concentrations near the usually applied legal limits for enology. PMB efficiency was also studied in red wines naturally contaminated by *Brettanomyces*. Volatile phenols levels were quantified after long aging of the wines treated with PMB. The results obtained showed that there is a negative correlation between the concentrations of PMB employed and the concentrations of 4-ethylphenol, 4-ethylguaiacol, 4-propylguaiacol, and *Brettanomyces* populations in the studied wine. For wine industry it was important that it was verified that 100 mg/L of total PMB could prevent efficiently *Brettanomyces* growth in the storage red wines and that volatile phenols concentrations were significantly ($P < 0.05$) higher in wines with lower levels of PMB.

5.2 Chitosan

Chitosan is a chitin-derived polysaccharide extracted from crustaceous or fungi species. Chitosan, the deacetylated form of chitin, is an important polyssaccharide because of the high density of amino groups present. In 2011, the use of chitosan in winemaking has been authorized by the EU (Reg. EU 53/2011) for heavy metals, iron and copper cloudiness (100 g/hL) contaminant removal, special ochratoxin A (500 g/hL), and for control and reduction of undesirable *Brettanomyces* spp. population (10 g/hL).

The antimicrobial activity of chitosan has been mainly explained by its positive electrical charge, which is incremented at acid pH and with higher chitosan degrees of deacetylation, so, a higher positive charge densityis directly correlated with antimicrobial effectiveness of chitosan (Kong et al., 2010). Portugal et al. (2014) showed that chitosan inhibitory effect against *B. bruxellensis* was identical in the presence or absence of 12.5% ethanol in the culture broth, where 62 mg/L of chitosan were enough to reduce 90% of yeast wine population (MIC50 and MIC90 were defined as the MIC, minimal inhibitory concentration, that inhibited 50 and 90%, respectively, of the experimental microorganisms, where MBC50 and MBC90 means minimal biocidal concentration). In the presence of 12.5% of ethanol, the MIC50 was also 62 mg/L. The MBC90 value was not obtained, because it was out of range of chitosan concentrations used in this work. However, Gómez-Rivas et al. (2004) reported that it is necessary to use 6 g/L of shell crab chitosan to completely inhibit *Brettanomyces* growth.

5.3 Dimethyl dicarbonate

Esters of pyrocarbonic acid, diethyldicarbonate (DEDC) and dimethyldicarbonate (DMDC), are known since 1938 (Boehm and Mehta, 1938). DMDC due to this lower toxicicity is usually prefered instead of DEDC, which releases ethyl carbamate from its degradation

(Schlatter and Lutz, 1990). DMDC is not a precursor of ethyl carbamate in wine, and therefore does not present a potential carcinogenic risk. When 200 mg/L of DMDC were applied to the wine, aromatic constitution was not altered (Ough, 1975). The hydrolysis of DMDC in wine is fast: 1 h at 30°C and 5 h at 10°C (Delfini et al., 2002). The legal limit in the United States, Australia, and European Union is 200 mg/L for several beverages (Costa et al., 2008). The antimicrobial activity of DMDC is particularly effective when the pH is low, and/or the concentration of DMDC and the temperature are high (Ough and Ingraham, 1961). The microorganism's inhibition occurs prior to complete hydrolysis. The presence of SO_2 also increased the antimicrobial power of DMDC, even though the combination of SO_2 and other preservatives (such as sorbic acid) reduced the effect of each component (Terrel et al., 1993). When DMDC is hydrolized, this inhibits acetate kinase and L-glutamic acid decarboxylase enzymes, and the enzymes alcohol dehydrogenase and glyceraldehyde-3-phosphate-dehydrogenase by methoxycarbonylation of the nucleophilic residues (imidazoles, amines, thiols) (Renouf et al., 2008). These authors also showed that 150 mg/L is enough to inhibit *B. bruxellensis* strains in different points of wine production: before and during alcoholic fermentation, before MLF, and in finished wine. In the prebottling treatment, 200 mg/L was also efficient. Due to the need for specific dosing equipment, proper safety and operator training, and maintaining the guarantee of a constant dosing, applying DMDC to wine harbors significative cost for the industry (Boulton et al., 1996). Currently, the wineries rent specialized equipment, as it is less expensive than buying them.

5.4 Weak Acids

Weak acids, such as sorbic and benzoic, are used extensively as preservatives in the food industry, and their effects on the efficiency of thermal treatment are considered of outmost importance (Guerzoni et al., 1990). Hydroxycinnamic acids (HCAs) may be also considered as weak organic acids (Rosazza et al., 1995), and thus, their inhibition mechanism may be postulated to resemble that of more common weak organic acids, such as acetic acid or lactic acid. Under acidic conditions, undissociated weak acids, in general, diffuse through the lipidic plasma membrane, reaching the more alkaline environment of the cytoplasm where dissociation occurs. Damage to the cells follows, unless the pH is reestablished by the out-pumping of H^+, with energy dissipation (Warth, 1988).

HCAs are endogenous compounds of grapes and are considered natural food preservatives, besides being precursors of volatile phenols. HCAs have been reported to inhibit the growth of a variety of organisms, including plants, fungi, and bacteria (Campos et al., 2003). The viability and intracellular pH changes in *B. bruxellensis*, in response to extracellular pH, as well as to the presence of an energy source and HCAs, have been investigated by means of fluorescent ratio imaging microscopy (FRIM) (Campolongo et al., 2014). The results obtained by the authors show that *B. bruxellensis* is able to maintain viability and increase its pH gradient with decreasing external pH values, whereas it is not able to maintain a pH gradient

at high external pH values (i.e., pH 8) and, as a consequence, dies. The growth inhibitory effects of ferulic and *p*-coumaric acid do not seem to be caused by a weak-acid inhibition mechanism, since both acids induce a similar, or even higher, intracellular acidification at a high external pH than at a low external pH. However, the results presented have to be confirmed by using other strains of *B. bruxellensins* in order to validate the outcomes obtained in these studies.

5.5 Enological Tannins

It is usually accepted that tannins can react with proteins, provoking precipitation of large macromolecular aggregates in wine, mostly established by hydrophobic interactions and hydrogen bonds. The antimicrobial activity of the tannins is related with those characteristics, that is, it increased in the presence of ethanol (Portugal et al., 2014), once ethanol changed the dielectric constant and modified hydrogen bonds, thus provoking cell flocculation and changing the membrane properties (Soares, 2011). The presence of 12.5% ethanol in the culture broth decreases substantially the quantity of tannins required to inhibit 50 and 90% of *B. bruxellensis* strains (Portugal et al., 2014). Because of this, both MIC50 and MIC90 (MIC50 and MIC90 were defined as the MIC that inhibited 50% and 90%, respectively, of the tested microorganisms, and an analogous definition was used for minimal biocidal concentration, MBC50 and MBC90), lowered, respectively, from 0.5–1 to 0.12 mL/L. Tannins showed large biocidal activity affecting 50% of the tested strains at a dosage of 0.25 mL/L in the presence of 12.5% ethanol; however, the MBC90 value was above the legal limit (0.4 mL/L) for the application of commercial enological tannins against *B. bruxellensis* in winemaking.

5.6 Reduction of Ethylphenol Precursors in Red Wines via the Formation of Pyranoanthocyanins

Brettanomyces is able to produce from grape HCAs the vinylphenol (VPh) and ethyl phenols (EPs) in two enzymatic steps. First the HCAs are decarboxilated to vinylphenols by hydroxycinnamate decarboxilase (HCDC) enzyme, which is reduced to ethyl phenols by vinylphenol reductase (Chatonnet et al., 1995) (Figs. 9.7 and 9.8).

It is possible to reduce precursors of EPs using HCDC + *Saccharomyces* yeasts during fermentation to release VPs (Suárez-Lepe and Morata, 2012). These compounds are able to rapidly react with grape anthocyanins forming vinylphenolic pyranoanthocyanins (VPAs) (Morata et al., 2006). VPAs are stable pigments under winemaking conditions (Bakker and Timberlake, 1997), decreasing at the same time the amount of EP precursors. There are only a few amounts of hydroxycinnamic in grapes, as free acids or sterificated with tartaric acid, these last ones can be slowly hydrolyzed in acidic polar media as wine. HCAs can increase during the barrel ageing raising the precursors for EPs. Natural hydrolysis can be accelerated using prior-to-fermentation cinnamyl esterases enzymes. These enzymes rapidly release

HCAs, then HCDC + *S. cerevisiae* strains transform HCAs in VPs by an enzymatic process. At the end, VPs are able to react chemically with grape anthocyanins yielding VPAs. This enzymatic-biological–chemical mechanism globally reduces the precursors for EPs (Morata et al., 2013).

5.7 Pulsed Electric Field (PEF)

Pulsed electric field (PEF) technology constitutes one of the most novel technologies for the pasteurization of foods due to its ability to inactivate pathogenic and spoilage microorganisms at room temperature, without modifying the quality of food (Martín-Belloso and Sobrino-López, 2011). Strong electric fields ranging from 26 to 35 kV/cm are produced in 1–4 μs pulses between two electrodes contacting the product (Garde-Cerdán et al., 2007). Short-duration high-intensity field strengths cause the electroporation of cell membranes and an increase in their permeability (Zimmerman, 1986). Cell death is achieved as dielectric breakdown increases permeability of the cytoplasmic and nuclear membranes, leading to cell lysis (Puértolas et al., 2010). Although PEF processing is effective against most microorganisms, yeasts tend to be more sensitive than bacteria (Marselles-Fontanet et al., 2009). PEF has been investigated as a means to reduce microbial contamination in wines (Santos et al., 2012). Puértolas et al. (2009a,b) reported that PEF treatment reduced populations of *B. bruxellensis* by 99.9% in both grape must and wine. Among yeast, *B. bruxellensis* in must and *S. bayanus* in wine were the most sensitive microorganisms, and *D. anomala* was the most PEF resistant independently of the medium. The wine obtained from PEF-treated grapes was similar to the control wine from a sensory point of view (Puértolas et al., 2010).

5.8 Low Electric Current (LEC)

Low electric current (LEC) is a treatment that involves the application of direct current voltage pulses for very short periods of time, in the range between microseconds to milliseconds, through a material placed between two electrodes. This voltage results in an electric field, the intensity of which depends on the gap between the electrodes and the voltage delivered (Puértolas et al., 2012). The possibility of applying low-intensity electricity has been studied because of its effects on viable microflora and related microbial interactions (Bawcom et al., 1995; Rajnicek et al., 1994; Shi et al., 1993). In fact, the application of external electric fields to biological cells causes alteration in the membrane structure, leading to pore formation. Under mild pulsation conditions, membrane pore formation is reversible, whereas more drastic conditions lead to the irreversibility of the phenomenon that eventually results in cell death (Weaver and Chizmadzhev, 1996). Current is generally applied to the product at less than 200 mA over the period of several days to several months (Lustrato et al., 2010; Palaniappan et al., 1992). Microbial inactivation is caused by electrical breakdown in the membrane's lipid bilayer (Lustrato et al., 2003). Increasing this current resulted in

a direct relationship with reduced membrane integrity and metabolic activity (Lustrato et al., 2003; Ranalli et al., 2002). Lustrato et al. (2010) applied LEC to contaminated red wine and concluded that a 200 mA treatment over a 60-day interval reduced *B. bruxellensis* populations by over six logs. The effects of LEC in controlling yeast spoilage were found to be comparable with those of adding SO_2. Lustrato et al. (2006) reported that wine fermented using LEC was not organoleptically different from wine fermented using SO_2. In contrast, Nakanishi et al. (1997) observed that wine fermented using a 100 mA treatment had overall greater final concentrations of higher alcohols, esters, some organic acids, and acetaldehyde.

6 Reduction of 4-Ethylphenol and 4-Ethylguaiacol Using Fining Agents

6.1 Classic Enological Products: Bentonite, Activated Carbon, and PVPP

Fining agents have to be removed from wine as they are not additives but technological adjuvants. Most fining agents react within seconds and the contact time between the fining agent and the wine should be as short as possible. Activated carbon and PVPP can be filtered out immediately or a few hours after fining. At the opposite, formation of flocculates requires a few days when proteins are used (depending on wine temperature) and they require a week or two to settle. If the electrical charge is opposite of the particles in suspension, neutralization and adsorption may occur. In most cases, the fining agent adsorbs the suspended material and exerts some clarifying action by virtue of formation of particles of high density, thus increasing filterability (Marchal and Jeandet, 2009). Some enological products, such as bentonite, PVVP, and activated carbon, used at many years in the wine industry, could remove partially the "Brett character" from wine (Table 9.2).

6.2 Yeast Cell Walls

Yeast cell walls, yeast hulls, and lees have been used in wine as biosorbents of undesirable molecules. Sites for the interaction between different molecules and yeast are located on the yeast wall. Yeast walls may bind undesirable compounds present in must that are toxic for cells and they might provoke a stuck fermentation (medium-chain saturated fatty acids) (Lafon-Lafourcade et al., 1984).

The yeast cell wall is composed of a three-dimensional internal skeletal layer of 1,3-β-glucan and 1,6-β-glucan (30%–40% of wall mass) stabilized by hydrogen bonds. Other important components of the yeast cell wall are the mannoproteins (30%–40% of wall mass), which are the most highly exposed cell-wall molecules, and these may therefore form sorption sites (Pradelles et al., 2008). 4-Ethylguaiacol and 4-ethylphenol show a fast sorption process, indicating that they become fixed to the functional groups of the molecules that cover the surface of the yeast walls (Nieto-Rojo et al., 2014). These authors showed, in synthetic wine, that the sorption ability of yeast cell walls for 4-EG is greater and with a faster kinetics than

Table 9.2: Reduction of ethylphenols by activated carbon, PVPP, and yeast cell walls.

Enological Product	Dosage (g/hL)	Matrix	Before Treatment		After Treatment (μg/L, %)		Analysis Methods	Authors
			4-EP	4-EG	4-EP	4-EG		
Activated carbon	20	Red wine	539	46	442 (18%)	41 (11%)	n-Pentane, GC/MS-SIM	Lisanti et al. (2008)
Activated carbon	80	Red wine	1500	300	58%	56%	n-Pentane/ diethyl ether, GC/MS-SIM	Milheiro et al. (2016)
Activated carbon	20	White wine	2470	250	1803 (27%)	185 (26%)	n-Pentane, GC/MS-SIM	Lisanti et al. (2008)
PVPP	80	Red wine	539	46	481 (11%)	—	n-Pentane, GC/MS-SIM	Lisanti et al. (2008)
PVPP	50	Red wine	1500	300	4.4%	—	SPME, GC/ MS-SIM	Milheiro et al. (2016)
Yeast cell walls	500	Synthetic wine	—	—	21.7 (%)	47.8 (%)	SPE, GC/MS	Nieto-Rojo et al. (2014)
Yeast cell walls	500	Synthetic wine	1000	1000	920 (8%)	956 (4.4%)	SPE, GC/MS	Jiménez-Moreno and Ancín-Azpilicueta (2009)

PVPP, Polyvinylpolypyrrolidone.

for 4-EP; however, the bonds are weaker than in the case of 4-EP. The capacity of yeast cell wall on reduction or mitigation of 4-EP and 4-EG can be observed in Table 9.2.

6.3 Cellulose Acetate

The complex carbohydrate cellulose, being the basic structural component of the plant cell wall, is the most abundant polymer in nature. In the primary structure, glucose units are attached together by β-1,4 linkages forming a long chain (microfibril). These chains are able to form intramolecular hydrogen bonds together, producing a cellulose fiber with unique properties in terms of mechanical strength and chemical stability, and also able to form a large number of hydrogen bonds on its surface, along with other molecules. The substitution of acetyl, propyl, and butyl groups for OH cellulose groups leads to materials, such as cellulose acetate (CA; CAS 9004-35-7), cellulose acetate propionate (CAP; CAS 9004-39-1), cellulose acetate butyrate (CAB; CAS 9004-36-8), and cellulose propionate (CP; CAS 9004-48-2). The presence of acetyl, propyl, and butyl groups leads to cellulose derivatives that can be effectively dissolved by solvents, such as acetone (Allen and Bevington,

1989). The ability of cellulose acetate, cellulose acetate propionate (CAP), cellulose acetate butyrate (CAB), and cellulose propionate (CP) fibers to reduce 4-ethylphenol and 4-ethylguaiacol was tested by Larcher et al. (2012). CAP and CP performed best in a preliminary comparative test. CAP effectiveness was studied in relation to the reduction in volatile phenols with doses of up to 20 g/L and wine contact times of up to 60 min. Using 4 g/L, reduction of both phenols was, on average, 31%–32% in defective red wines. Wine treatment affected neither color nor total proanthocyanidins and catechins, and wines were judged to be better than the corresponding spoiled controls. CAP fiber can be regenerated by washing with ethanol or aqueous solution (pH 12), without notable changes in depletion efficiency. The technological characteristics of CAP and, to a lesser extent, CP offer interesting prospects for producing filtration beds, pads, and membranes for the treatment of wines and beverages affected by "Brett character".

6.4 Polyaniline-Based Materials (PANI-EB and PANI-ES)

One group of compounds with potential applications in several fields, including the removal of unwanted substances from food and beverages, is the polyaniline (PANI)-based materials. PANI has been used in the development of transistors, switches, electrochemical actuators, and lightning protection (Marican et al., 2014). The polyaniline-based polymers exist in several forms, the most stable of which are the emaraldine states (i.e., emeraldine salt, ES and emeraldine base, EB). They have been synthesized in high yields in aqueous medium, are stable at high temperatures and pH, and are not metabolized by common microorganisms (Ashley et al., 2011). The reason is possibly based on the fact that PANI has often been regarded with caution because its reaction intermediates (aniline, dimers, and oligomers) are aromatic amines that can be physiologically active or even harmful (Yslas et al., 2012). PANI is relatively inexpensive, easy to synthesize, has good environmental stability, and shows interesting redox properties associated with the nitrogen chain. The ability of polyaniline-based materials (PANI-EB and PANI-ES) was tested as a potential fining agent for the removal of 4-EG in wine. First, a screening study was developed in order to determine the binding capacity of 4-EG by PANI materials in 12% ethanol solution. Then, the capturing ability of PANI against 4-EG was evaluated with a solution containing gallic acid (GA), 4-methyl-catechol (4-MC), in which the concentration of the phenolic compounds were maintained as in the real wine. The results obtained by Yslas et al. (2012) showed that the retention percentage varied from 0% to 100% (4-EG), 13.81% to 72.32% (GA), and 0% to 17.39% (4-MC), depending on the interaction time and amount of the PANI used. Finally, the capturing capacity of PANI-EB and PANI-ES against 4-EG was evaluated in a real wine sample containing originally 3.10 mg/L of 4-EG and 2.55 g/L of other total phenolic compounds. The analysis performed indicated that PANI-EB is more effective in removing 4-EG than PANI-ES, with retention percentages varying between 36% and 50%.

6.5 Cyclodextrins

Cyclodextrins (CDs) are carbohydrates composed of α-1,4-linked glucopyranose, also called glucose units, where the glucopyranose molecules are arranged to form a ring that looks like a bottomless bucket (Botelho et al., 2011). The outer side of this ring has a molecular structure that gives it solubility in water (as other simple carbohydrates), while the inside repels water, favoring molecules that are insoluble in water. These molecules, dissolved or dispersed in water, have a preference for being in the cavity rather than in water (Duchene et al., 2003).

When the host molecules are in the cavity, it is not covalent forces that prevent them from leaving, but atomic (Van der Waals), thermodynamic (hydrogen bonding), and solvent (hydrophobic) forces in the hydrophobic environment of the CD cavity (Astray et al., 2009). These links can be destroyed easily by heating, pH changing, and other factors. A complex can be formed if a balance exists between the relative concentrations of the CD and the potential guest chemical. The rate at which the associated complex is formed is determined by the accessibility of the guest molecule to the CD cavity and the magnitude of the thermodynamic driving force. Hydrophobic guest molecule is removed from the aqueous environment, while water molecules previously associated in the CD cavity return into aqueous environment; that's why this binding is energetically favorable (Singh et al., 2002).

According to these preliminary results obtained by Botelho et al. (2011), the concentration of 11.52 g/L of β-CD (ratio of 2:1) was enough to remove the off-odors caused by 4-ethylphenol or 4-ethylphenol + 4-ethylguaiacol in a red wine by sensorial analysis. Furthermore, the addition of β-CD to red wine increased significantly the color intensity and the total polyphenols index.

7 Prevention of Biogenic Amines Formation During Winemaking

As mentioned earlier, BA origin and levels in wines is a multifactorial problem, starting from agricultural practices to the winemaking technology (Ancín-Azpilicueta et al., 2008; Del Prete et al., 2009). In order to reduce the presence of BA in vine-based products, the International Organization of Vine and Wine (OIV) published the "OIV Code of Good Vitivinicultural Practices." According the OIV Guide (2011), the actions in vineyards and cellars favoring the presence of BA in wines are: soil, nitrogenous fertilization, poor state of health of the grapes combined with mold, a high must pH, and development of certain yeasts during alcoholic fermentation. Also the levels of BA in wines are significantly increased by LAB during MLF (Ancín-Azpilicueta et al., 2008). According to Callejón et al. (2014), there are three main ways of diminishing the BA formation: preventing the growth of spoilage bacteria, reducing the concentration of amino acid precursors, and inoculating starter cultures without amino acid decarboxylases.

7.1 Methods for Reduction of Formed BA in Wines

Fining of finished wines with bentonite, either calcium, sodium-calcium, or sodium form are able to reduce the content of BA in wines (Binner et al., 2013; Grossmann et al., 2007; Kallay and Body-Szalkai, 1996; Mannino et al., 2006). The efficiency of removal was dependent on the bentonite dose used with a reduction of 40% at 100 g/hL of bentonite for a BA content of 25 mg/L (Mannino et al., 2006), nevertheless high bentonite doses can impact negatively on the sensorial and chromatic characteristics of wine. To overcome this problem, the previous addition of ellagic tannins to wine can decrease the amount of bentonite needed (reduction to 20 g/hL obtaining the same results of 60 g/hL of bentonite only) (Mannino et al., 2006). Nevertheless, when fining is applied to the must, the decrease of BA in the final wine is higher (80% decrease for must treatment compared to 40% obtained for the wine treatment (Binner et al., 2013; Grossmann et al. 2007), and besides the decrease observed for bentonite, other fining agents, such as potassium caseinate, gelatin, and activated carbons also have an effect on the BA levels in the final wine (Grossmann et al., 2007). Recently the use of commercial zirconium phosphate in the sodium form showed strong adsorbent activity for histamine and putrescine (Amghouz et al., 2014). The use of material with high selectivity for the BA can be a good technology to reduce the BA levels in wines after the fermentation.

7.2 Methods for Degrading Formed BAs

Another alternative for the reduction of BA levels in wines is its degradation by the use of microorganisms or enzyme preparations. Garcia-Ruiz et al. (2011) studied the ability of diverse LAB strains for their ability to degrade hitamine, tyramine, and putrescine. They observed that 25% of the strains were able to degrade histamine and 18% were able to degrade tyramine and putrescine. Two *L. plantarum* biotypes of twenty-six strains were able to synergistically reduce BA levels with higher efficiency (Capozzi et al., 2012). This reduction of BA by bacteria was attributed to the presence of amine oxidase activity. The use of enzyme preparation presenting amine oxidase activity instead of the cells has been also studied (Callejón et al., 2014; Sekiguchi et al., 2004), although their efficiency has been limited by the low wine pH and also the complex wine matrix.

8 Ethyl Carbamate Mitigation

The presence of ethyl carbamate in wines is mainly related to the formation of urea by yeasts, where its excretion and utilization during alcoholic fermentation is governed mainly by the yeast strain used and environmental conditions, that after reaction with ethanol results in this potential toxic product (Ough et al., 1988b). So, important parameters for the postfermentative formation of ethyl carbamate are the concentration of ethanol, urea, citrulline, temperature, and time of storage (Hasnip et al., 2004). A less important parameter is the levels of carbamyl

phosphate produced by yeasts, and in addition, daylight exposure doesn't seem to be related with ethyl carbamate formation (Tegmo-Larsson and Spittler, 1990). At present, only preventive treatments have been found to be efficient for the reduction of ethyl carbamate levels in wines. Principal among these has been a series of preventative actions drawn from scientific research and produced jointly by U.C. Davis, the Wine Institute, and the US Food and Drug Administration (Butzke and Bisson, 1997). These actions covering best practices in viticulture, juice nutrient status, yeast strains and LAB, urease application, *sur lie* aging, distillation, and shipment and storage have helped US wine growers and distillers to control the formation of ethyl carbamate in their products. On the other hand, legislation authorizes the use of an enological additive, the enzyme urease, in wines with high urea levels. The urease used is isolated from *L. fermentum*. This enzyme has been effective on urea at doses of 50 mg/L in red wines and at doses of 25 mg/L in white wines (Bertrand, 1997).

9 Application of Fining Agents to Reduce OTA Concentration from Wine

According to the European Commission Regulation (EC) No. 123/2005 the maximum limit for OTA in wine is 2 μg/kg [Commission Regulation (EC) No. 123/2005]. Therefore, it is important to prevent and control their occurrence in wines. In general red wines comprise greater OTA concentration than white, which is related with the vinification process of red wines, namely with the maceration operation. With the purpose to remove this toxin, several adsorvents (potassium caseinate, activated carbon, bentonite, egg albumin, gelatin, and polyvinylpolypyrrolidone) have been described in the literature by several researchers, such as Castellari et al. (2001), Kurtbay et al. (2008), Quintela et al. (2012a, 2012b), in order to study the removal of OTA from contaminated wine. The application of the different fining agents aims to assess their ability to firmly bind and immobilize the mycotoxin**.** The outcomes obtained in the different studies showed that the efficiency in the removal of OTA from the wine is influenced by several factors, such as the wine OTA concentration, the fining agent applied, in addition to its type and dosage.

10 Final Remarks

Yeast and bacterial meeting the best enological characteristics and the optimization of fining and technological treatments are the future steps to produce quality wines, promoting human health and enjoyment, with benefits to the palatability of wines.

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