DOI 10.1007/s00217-013-2036-4

Impact of volatile phenols and their precursors on wine quality and control measures of *Brettanomyces/Dekkera* yeasts

Joyce Kheir · Dominique Salameh · Pierre Strehaiano · Cédric Brandam · Roger Lteif

Abstract Volatile phenols are aromatic compounds and one of the key molecules responsible for olfactory defects in wine. The yeast genus *Brettanomyces* is the only major microorganism that has the ability to covert hydroxycinnamic acids into important levels of these compounds, especially 4-ethylphenol and 4-ethylguaiacol, in red wine. When 4-ethylphenols reach concentrations greater than the sensory threshold, all wine's organoleptic characteristics might be influenced or damaged. The aim of this literature review is to provide a better understanding of the physicochemical, biochemical, and metabolic factors that are related to the levels of *p*-coumaric acid and volatile phenols in wine. Then, this work summarizes the different methods used for controlling the presence of *Brettanomyces* in wine and the production of ethylphenols.

Keywords Wine · *p*-Coumaric acid · 4-Ethylphenol · Bioconversion · *Brettanomyces/Dekkera*

Introduction

The fermentation process of winemaking is the result of a complex biological and biochemical interaction between yeasts, bacteria (lactic and acetic), and filamentous fungi present in grapes, grape musts, and wine at various stages. If the wine flavor is directly determined by grape variety, microorganisms can also affect it by the production and excretion of metabolites during growth and through autolysis [1]. The involvement of microorganisms in wine must be controlled in order to avoid any negative impact on the organoleptic quality of the final product.

In some cases, the growth of some yeasts such as *Brettanomyces* species can result in wine defects and subsequent spoilage. Depending on the conditions and on the available precursors (hydroxycinnamic acids, also called phenolic acids), this yeast genus can produce undesirable metabolites when growing and/or aging in wine. These phenolic acids may be advantageous by inhibiting microbial growth [2] and are known to be precursors of volatile phenols [3, 4]. In the wine production field, the term "volatile phenols" usually means the group of compounds made up of 4-ethylphenol, 4-ethylguaiacol, and 4-ethylcatechol. Depending on their concentration levels, volatile phenols can be considered as normal constituents or as the cause of deterioration of wine quality. At low levels, some winemakers accept the presence of these products, which can greatly give a distinctive aged character to some young red wines. It favorably contributes to the complexity of aroma by imparting aroma notes of spices, smoke, and leather [5] and by giving good sensory attributes of bitterness and astringency [4]. When the levels of these low molecular weight volatile phenols exceed the perception threshold (0.047 mg/l for 4-ethylguaiacol and 0.23 mg/l for 4-ethylphenol) [6], *Brettanomyces* can potentially induce spoilage related to medicinal or barnyard odors. Moreover, *Brettanomyces* is responsible for phenolic off-flavors (POF) of smelling mousy, horse sweat, or rancid cheesy aroma; isovaleric acids are known to be

J. Kheir (✉) · P. Strehaiano · C. Brandam
Laboratoire de Génie Chimique, Université de Toulouse, INPT, UPS, 4, Allée Emile Monso, 31030 Toulouse, France
e-mail: joyce.kheir@ensiacet.fr

J. Kheir · P. Strehaiano · C. Brandam
Laboratoire de Génie Chimique, CNRS, 31030 Toulouse, France

J. Kheir · D. Salameh · R. Lteif
Faculté des Sciences, Université Saint-Joseph, Mar Roukoz, Mkallès, P.O.Box 11514, Beirut 1107-2050, Lebanon

responsible for that cheesy aroma, while mousy odors are the result of tetrahydropyridines (2-ethyltetrahydropyridine, 2-acetyltetrahydropyridine, and 2-acetylpyrroline) [7] synthesized by *Brettanomyces bruxellensis* from lysine and ethanol. The off-flavors can appear in all red wines at different production stages, mainly in aging processes [8], prior to bottling, especially when wines are stored in barrels, particularly old barrels, if rarely or never racked. This organoleptic contamination, described as the phenolic character or "Brett character," leads to consequent economic loss. Among all the POF that *Brettanomyces* genus can produce, the production of 4-vinylphenol and 4-ethylphenol from *p*-coumaric acid is certainly the most predominant reaction in terms of quantity and frequency. This review presents a complete description of these reaction mechanisms and then recapitulates different factors that directly affect them. Two types of factors are discussed: first, physicochemical factors that can directly influence the amount of *p*-coumaric acid present in the medium before bioconversion takes place or the amount of 4-ethylphenols already formed. Then, this review cites various biochemical and metabolic factors that affect the bioconversion and can effectively modify the kinetics of the reaction. Finally, this work attempted to include some aspects of control of *Brettanomyces* growth; thus, understanding the physicochemical attributes of growth is fundamental to understanding how to achieve effective control of the organisms.

Main characteristics of *Brettanomyces/Dekkera* yeasts

In 1904, the name *Brettanomyces* was proposed for the first time by N.H. Claussen for yeast involved in the secondary fermentation of the so-called English stock-beer. He included these strains in the genus *Torula*. The name *Brettanomyces* was first used, in 1921 by Kufferath and Van Laer, as a generic name for two yeasts (*Brettanomyces bruxellensis* and *Brettanomyces lambicus*) isolated from lambic beer and which showed much resemblance to Claussen's strains [9].

In terms of morphology, *Brettanomyces* yeasts are pyriformis ("ogive"-shaped, more or less elongated) with multipolar budding (multilateral). They are also known to form pseudomyceliums. In sexual reproduction, they present ascus containing one to four ascospores [10]. However, the morphology of *Brettanomyces* sp. may change depending on environmental conditions [11]. This may explain the difficulties in their detection in alcoholic beverages. Indeed, under stressful conditions, they become smaller, to the point of passing through the membrane of 0.45 μm, while the normal size of *Brettanomyces* sp. varies from 2 to 7 μm. In this form, they are part of the flora viable but not cultivable, but may resume normal growth after an enrichment culture of a week [12].

Optimum range of growth conditions

Brettanomyces has been isolated from the outside of grapes and from winery equipment more commonly in vats, pumps, and on materials that are difficult to clean. It can also grow during red wine aging in oak barrels, particularly when SO_2 concentration is low (molecular $SO_2 < 0.5$ mg/l), pH is high (>3.8), and temperature is above 15 °C [13], and sometimes even after bottling. The porous microstructure of (new and old) oak barrels allows the influx of small amounts of oxygen, which helps the growth of *Brettanomyces* [14]. The use of old wooden casks can also increase the presence of these species because they are impossible to sterilize.

Brettanomyces is known to be as a slow-growing yeast (naturally slower than *Saccharomyces*) taking over a week or several weeks to reach a detectable population size and that does not compete well against other microorganisms. Most of the strains could grow on any of the monosaccharides glucose, fructose, and galactose or the disaccharides sucrose, maltose, cellobiose, and trehalose [15], and when grown on glucose-rich media, it produces large amounts of acetic acid. It can also proliferate under warm cellaring conditions such as high alcohol levels and the existence of even small amounts (<300 mg/l) of fermentable sugars such as glucose, which is its preferred source of energy for growth. Several observations show that glucose exerts a repression or inhibition system assimilation of other sugars. Thus, in a mixture of cellodextrins–glucose, preferential consumption of glucose is expected [16]. To make matters worse, *Brettanomyces custersii* and *Dekkera intermedia* metabolize cellobiose, a disaccharide forming the basic repeating unit of cellulose (a structural polysaccharide of wood) [17].

Brettanomyces yeasts usually do not require nutrition-rich environments for their growth. It has been reported that this genus of yeast is the only microorganism that can survive in wine after bottling, due to its ability to resist in the anaerobic conditions [18]. Thus, *Brettanomyces* showed good biomass production in semi-aerobic and anaerobic conditions, while a reduction in both growth rate and biomass concentration was shown in strict-anaerobic conditions. In this latest condition, the amount of dissolved oxygen in wine at the beginning of fermentation was sufficient to stimulate the growth and the fermentation. In fact, the stimulation of alcoholic fermentation by an H-acceptor such as oxygen has been considered as a biochemical characteristic of *Brettanomyces/Dekkera* (Custers effect) [19].

Under fully aerobic conditions, *Brettanomyces* yeasts are able to multiply faster and can produce large amounts of acetic acid while ethanol concentrations were found to be lower. Under the same conditions, *Brettanomyces* has

been shown to display a loss of viability after 200 h [20]. The presence of oxygen is known to reduce the sensitivity of *Brettanomyces* to SO_2, but strain variations also influence this sensitivity [21]. On the other hand, semi-aerobic conditions cause a decrease in the production of acetic acid. In addition, *Brettanomyces* is tolerant to high levels of ethanol (14.5–15 %) [22]. Also, the addition of ammonium sulfate or yeast extract to the medium is thought to be as favorable conditions for their growth [11]. Similarly, vitamins such as biotin and thiamine can positively affect the growth of this organism [15]. Also, growth of *Brettanomyces bruxellensis* can be stimulated by the addition of ammonium sulfate or yeast extract to the medium [11]. Concerning the concentrations of SO_2, data shown in literature were different. Du Toit et al. [21] reported that *Brettanomyces* is sensitive to the levels between 0.25 and 0.35 mg/l of molecular SO_2, while Barata et al. [23] recommended an adjustment of its level to 1.0 mg/l in wine before barrel aging.

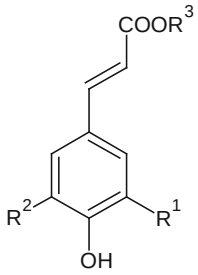
Bioconversion of hydroxycinnamic acids into ethyl derivatives by *Brettanomyces*: main enzymatic reactions involved

Phenolic compounds in wine are generally divided into two groups, flavonoids and non-flavonoids [24, 25], based on their carbon skeleton. A third group of phenolic complexes has been reported by Basha et al. [26]. These compounds are known as phenolic-protein-polysaccharide complexes.

Flavonoids include anthocyanins, flavan-3-ol monomers and polymers, flavonols, and dihydroflavonols. The majority of non-flavonoids found in grapes are hydroxycinnamic acids (found esterified with tartaric acid, caftaric acid, coutaric acid, and fertaric acid); hydroxybenzoic acids (gallic acid, a hydrolysis product from grape or oak tannins), stilbenes (resveratrol and piceid), and phenolic alcohols [25]. The chemical structure of flavonoid compounds is based on 15 atoms of carbon, including two benzene rings joined by a linear three-carbon chain, while non-flavonoid structure presents one primary aromatic ring attached to either one or three carbons.

The different compounds present in wine are mainly derived from hydroxycinnamic acids, including caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid (Fig. 1). These derivatives can be present in *cis* and *trans* configured forms, while the *trans* forms are more stable and, therefore, more prevalent [27].

Most of the hydroxycinnamic acids are initially present in grapes. During the maceration process, they are extracted from the berries and pass into the grape juice. As fermentation begins, ethanol concentration becomes higher, thus increasing the extraction of all phenolic compounds. In wine, hydroxycinnamic acids are present in low amounts



	R^1	R^2	R^3	MW
Caffeic acid	OH	H	H	180
Caftaric acid	OH	H	Tartaric acid	312
p-Coumaric acid	H	H	H	164
p-Coutaric acid	H	H	Tartaric acid	296
Ferulic acid	OCH_3	H	H	194
Fertaric acid	OCH_3	H	Tartaric acid	326
Sinapic acid	OCH_3	OCH_3	H	224

Fig. 1 Structures of hydroxycinnamic acids in wine [27]

in their free form, while other forms such as esters of l-(+)-tartaric acid are predominant. The ubiquitous chlorogenic acids, esters of hydroxycinnamic and quinic acids, cannot be found in wine, but are replaced by tartaric acid esters [27]. The action of cinnamoyl-esterase enzymes releases these weak acids to their free forms [28], in which they can be inhibitory to the growth of many microorganisms. The origin of volatile phenols is related to the sequential activity of two enzymes that decarboxylate free hydroxycinnamic acid precursors (*p*-coumaric, ferulic, and caffeic acids) into hydroxystyrenes (4-vinylphenol, 4-vinylguaiacol, and 4-vinylcatechol), which are then reduced to their corresponding ethyl derivative forms (4-ethylphenol, 4-ethylguaiacol, and 4-ethylcatechol) [4]. The first enzyme is a cinnamate decarboxylase (analogous to the *Pad*1 enzyme of *Saccharomyces cerevisiae*), which cleaves the C3 (Fig. 2) carbon from the side chain releasing CO_2. The second, a vinylphenol reductase (VPR), reduces the double bond on the vinyl derivative to form the ethyl substitute compound [29]. The capacity of *Brettanomyces/Dekkera* spp. to form ethylphenols was demonstrated for the first time during the fermentation of a grape must [30, 31]. The name *Dekkera* describes the teleomorph (perfect state), which means the sporogenous form.

Recent studies show that *Brettanomyces* only metabolize vinylphenols and do not decarboxylate hydroxycinnamic acids [32]. A partial hydroxycinnamate decarboxylase (HcD) protein sequence from *Dekkera anomala* has been described. It does not share similarities with the previously sequenced HcD genes from other yeasts or bacteria. It does, however, align to the same

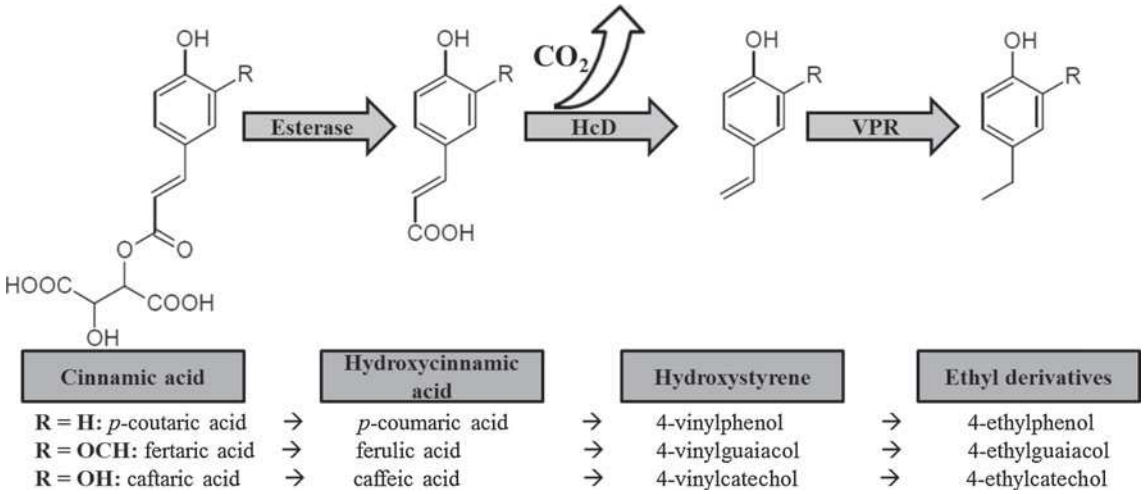


Fig. 2 Pathway of formation of volatile phenols via the decarboxylation of hydroxycinnamic acids

protein as the N-terminal sequence reported by Edlin [33]. This novel enzyme, found in *Dekkera* species, is responsible for hydroxycinnamic acid decarboxylation. In this previous work, the authors reported the lack of HcD in some species of this yeast and not as a general conclusion about the genus.

Decarboxylation reactions exist in numerous bacteria, fungi, and yeast species under enological conditions. The fermenting yeast *S. cerevisiae* is able to decarboxylate the side chain of hydroxycinnamic acids to produce vinyl derivatives, but is incapable of reducing them to critical concentrations of ethyl derivatives [4, 29, 31]. Other yeast genera that also decarboxylate *p*-coumaric and ferulic acid include *Rhodotorula*, *Candida*, *Cryptococcus*, *Pichia*, and *Hansenula* [34]. Some strains of *Lactobacillus brevis* and *Pediococcus pentosaceus* are also able to decarboxylate *p*-coumaric acid to form vinylphenols, but are unable to break down ferulic acid [34]. It is much rarer for lactic and acetic bacteria isolated from wine to synthesize significant quantities of 4-ethylphenol [35]. The reduction step and the production of large quantities of ethylphenols occur less frequently in microorganisms, but are particularly effective with the population of *B. bruxellensis*, *B. anomala* [36], and *B. intermedius* [37]. In grape must, strains of *Pichia guilliermondi* present a similarity with *Dekkera bruxellensis* strains in producing considerable quantities of ethylphenol [4]. As *P. guilliermondi* has been recovered from all winery equipment, they may have significance for wine spoilage through the production of volatile phenols. However, these species are not capable of producing high levels of 4-ethylphenol in wine as their viability is lost after 24 h of inoculation in red wine presenting normal conditions (pH 3.5 and ethanol concentration varying from 10 to 12 % v/v) and in the absence of free sulfite [38].

Factors affecting the production of 4-vinylphenol and 4-ethylphenol in wine

The formation of volatile phenols in wine depends on the presence of *p*-coumaric acid. Moreover, it is proportional to the size of *Brettanomyces/Dekkera* population [17, 39] and is also influenced by the vinylphenol enzymatic activity, which is specific to *Brettanomyces*. The influence of various strains of *B. bruxellensis* on the production of 4-ethylphenols and other volatiles has not been fully elaborated. The worldwide winemaking community is divided regarding the potential merits or deficiencies due to the growth of *Brettanomyces* in wine. It has been suggested that these differences may result from strain variations, which are well reported in the production of these compounds, length of time for *Brettanomyces*–wine contact, and the ratio of current to total cell exposure [40]. Other factors exist and may influence either the reactants and/or the products of the bioconversion reaction or even the reaction kinetics itself. The following paragraphs elaborate these factors which are divided into physicochemical components and biochemical and metabolic ones.

Physicochemical factors

Wine grape varieties and degree of maturation

A greater potential of volatile phenol production arises from higher levels of precursors (ferulic and *p*-coumaric acids) [41]. Thus, the availability of *p*-coumaric acid is essential for the significant production of 4-ethylphenol. Phenolic acids are normally found in small quantities in grapes, at the levels varying from traces to 15–20 mg/l in free forms and from 10 to 50 mg/l in bound forms.

However, the amount of these contents directly differs, based on the chosen grape variety [41–43]. According to the study of Pöllnitz et al. [44] on commercial bottled Australian red wines, pinot noir wines were reported to contain less *p*-coumaric acid, while commercial wines from cabernet sauvignon or merlot were the richest in this acid. Subsequently, low amounts of 4-ethylphenol formed resulted in pinot than the one formed in other types of grapes. This suggests a relation between concentration of precursors and products. On the other side, as for all cinnamic acids, white wines contain a concentration of *p*-coumaric acid <5–25 % compared to red wines, probably due to the lack of the maceration stage [45]. Red wines are also known to have a much higher level of coumaric and ferulic acid than white wines as they are extracted from the skins of grapes during red wine fermentation. Thus, *Brettanomyces* can produce 4-ethylphenol from 4-vinylphenol originally present in wine (action of *Saccharomyces* and different microorganisms), in addition to 4-ethylphenol produced by both steps of bioconversion from *p*-coumaric acid, initially present in wine [4].

Another factor affecting the initial quantity of *p*-coumaric acid in wine is grape maturity. This stage can be seen as a compromise between sugar content, total acidity, and aromatic or phenolic potential [46]. When grapes are allowed to ripen, phenolic maturity becomes more important; consequently, the quantity of phenolic compounds increases. Thus, the phenolic composition of wine is directly related to the decision concerning the date of harvest [47]. Indeed, grape at harvest time certainly contains a multitude natural microflora of bacteria, yeasts, and molds. The microbial population may be related to increased levels of POF. As an example, *Aspergillus* molds, which inhabit grapes and have esterases to hydrolyze phenolic acids bound to tartaric acids, can increase the free phenolic acid content in grape musts. This suggests the importance of sanitary conditions for wine grapes [41].

Process of extraction

The variation in 4-ethylphenol concentration between two red wines is partially due to the process of vinification [48]. One finding shows that for a wine made from the same grape variety and has undergone the same winemaking process, carbonic maceration has a direct influence on the amount of 4-ethylphenol which would appear in wine [48, 49]. Carbonic maceration generally takes place before alcoholic fermentation and leads to the production of small amounts of ethanol by the grape enzymes, in anaerobic metabolism and in an atmosphere saturated with CO_2. This maceration promotes a significant extraction of wine phenolic compounds (*p*-coumaric acid) [50, 51], especially when it happens at around 30–32 °C as high temperature favors extraction. Other physicochemical factors, such as restlessness, pumping, and concentration of SO_2 or CO_2, can also play an important role in the extraction process.

In fact, changing the temperature during winemaking processes may influence the extraction of phenolic compounds because temperature affects the permeability of cells and membranes in grape berries [52]. It was reported that higher anthocyanin content, color intensity, total polyphenols, and total sensory score were obtained in pinot noir wine by heating at the end of maceration [39, 53]. Moreover, heating at about 40 °C for 1 day after fermentation was preferable, as this temperature has a significant effect on phenolic extraction without producing a large quantity of volatile acid or oxidized off-flavor that is often reported under high temperature, for example, during a thermovinification process at 50–80 °C. Same authors did not observe any improvement in phenolic extraction as a result of cold soak, a vinification method in which wine musts are kept at low temperature for several days before alcoholic fermentation.

Adsorption of p-coumaric acid and ethylphenols

The *p*-coumaric acid, which may be naturally found under the form of esterified *p*-coumaroyltartaric acid, is partially lost in the physicochemical treatments for wine stabilization, particularly to offset tartaric instability. Tartaric acid is one of the main acids in wine. It may appear as either free or associated with other chemical compounds, including *p*-coumaric acid [54]. While studying bioconversion, Salameh et al. [55] explained the difference found among the initial concentration of the detected *p*-coumaric acid and the one added by the adsorption on *Brettanomyces* sp. when the population level increases. The same phenomenon was observed by Cabrita et al. [56] when a difference was found between the initial concentration (10 mg/l) of caffeic, ferulic, and *p*-coumaric acids added and the concentrations detected immediately after yeast inoculation. Other than adsorption on yeast cell walls, the loss of hydroxycinnamic acids in a synthetic medium might be explained either by their instability at high temperature or by esterification reaction with ethanol.

Furthermore, it is well known that during fermentation, yeasts may influence the color of wine [57]. They significantly reduce the composition of anthocyanin by adsorption on their wall [58] or through the intermediate periplasmic anthocyanin-β-D-glucosidase [59, 60]. In the same way, the yeast *S. cerevisiae* favors the formation of anthocyanin-vinyl derivatives between malvidin-3-O-glucoside and 4-vinylphenol. This may be different depending on the strains [61, 62]. It also reduces 4-vinylphenol from the medium, minimizing the risk of formation of 4-ethylphenol, on the one hand, and stabilizes pyranoanthocyanins

on the other hand [63, 64]. Indeed, vinylphenol derivatives with anthocyanins are very stable because of their aromatic heterocyclic ring [65, 66], which makes the effect of SO_2 minimal with the anthocyanin discoloration [65].

Other chemical reactions

The winemaking process leads to environments that always change. The deviation of organoleptic characteristics of wine is a direct result of variations in phenolic composition [67]. Indeed, the formation of weak or strong energy links between various phenolic compounds, on the one hand, and between polyphenols and other constituents of wine, on the other, plays an important role in enology [68–70]. As a result, *p*-coumaric acid can undergo a polymerization reaction or an electrophilic addition with other compounds, including S-glutathione. Similarly, 4-vinylphenol can undergo an electrophilic addition with alcohol in wine to give ethoxyethylphenols [71].

Several endogenous and exogenous enzymes of grapes can also act on *p*-coumaric acid, like other phenolic compounds during vinification, by either oxidizing or hydrolyzing these compounds. The main degrading enzyme of the grape polyphenols is polyphenoloxydase (PPO) which will oxidize the must and the future wine in an aerobic environment leading to a brown color. This phenomenon is known as an "enzymatic browning" [72, 73]. Thus, *p*-coumaric acid is an important substrate for polyphenol oxidase and can be oxidized enzymatically throughout the processing chain, even if PPO is primarily active at the beginning of vinification. Phenolic compounds can afford "auto-oxidation" in a non-enzymatic way under favorable conditions, but these reactions are limited and long, compared to enzymatic oxidation [74, 75]. In a hydro-alcoholic mixture solution, phenolic acids are colorless. They may become yellow after oxidation. But although *p*-coumaric acid induces a yellowish hue in wine [76], this compound does not appear to undergo a chemical browning [77]. Because of their antioxidant function, phenolic compounds are known to inhibit the development of various oxidations [78, 79].

Pectinases, β-glucanases, tannase, and cinnamate esterase are hydrolytic enzymes, by which hydrolysis of *p*-coumaroyltartaric acid in must releases *p*-coumaric acid in wine [80]. It should be noted that esterase activity may continue in the finished wine, which increases the amount of *p*-coumaric acid released from its ester residuals over time [43]. As many reactions can increase the quantity of *p*-coumaric acid in the medium, others can cause a decrease in the amount available for bioconversion as it was shown by Salameh et al. [55], which explained *p*-coumaric disappearance at the beginning of the reaction by an esterification reaction. This reaction is known to occur in all wine polyphenols.

It is noteworthy that ethylphenol in red wine is not stable and its concentrations, as well as the resulting sensory profile, can be influenced. This reaction is still poorly understood, whereas it is known that 4-ethylphenol can be broken down into 4-hydroxyacetophenone via the enzyme 4-ethylphenol methylene hydroxylase (4EPMH) and into 4-hydroxyacetophenone and 4-vinylphenol via the enzyme *p*-cresol methylhydroxylase (PCMH). In the same way, the breakdown products of 4-ethylguaiacol are 4-vinylguaiacol and 4-acetovanillone. The potential degradation products can influence the aroma profiles of wine as 4-hydroxyacetophenone has a sweet floral aroma; 4-vinylphenol has an almond shell aroma, 4-vinylguaiacol a clove-curry aroma, and 4-acetovanillone a vanilla aroma [81].

Aging wine types and effects of oxygenation

Maturation and aging can be considered as a group of reactions and series of changes that occur in wine during storage and lead to wine improvement. Wine aging should not be viewed as a single procedure and a single event, but rather as a series of changes. A distinction exists between the term maturation and aging. Maturation is used for changes during bulk storage, whereas aging is used for changes during bottle storage. The key difference is that during bulk storage, a wine is likely to be exposed to air, whereas it is stored in bottles in essentially anaerobic conditions. It was showed that the presence of volatile phenols is mainly due to the phenomenon of maturation [82]. In general, a time of breeding of nine months is enough to have a phenolic character affecting wine aromas.

Stages of aging wine are responsible for the enrichment of phenolic composition. Indeed, using oak barrels for wine aging is a respected tradition because wood releases the odorous constituents of a wine [83–85]. Oak wood is composed of four principal constituents: cellulose, hemicellulose, tannins, and lignins. Lignin makes up 25–35 % of the dry weight of wood and can be degraded by heat, hydrolysis, and microbial action. During seasoning and toasting, lignins undergo substantial chemical changes. Compounds that result from thermal degradation are vanillin, guaiacol, 4-ethylguaiacol, ferulic acid, 4-methylguaiacol, 4-ethylphenol, and *p*-coumaric acid. Extraction of volatile compounds from oak barrels depends mainly on the quantity of compounds that are potentially extractable, on the contact time between wine and wood, and on the wine composition [86].

Similarly to oak barrels, the oxygenation allows favorable development of polyphenols [74, 87, 88]. This quality of maturation made from wood entails several difficulties and risks.

Under the same conditions, wine reared in stainless steel vats has concentrations of 4-ethylphenols twenty to forty

times lower than those observed in barrels [6, 31], while these compounds were formed in a higher quantity when wine is stored in used barrels and their concentration tends to increase during aging. Ethylphenols were also formed in wine aged in new barrels. Their concentrations were lower after 6 months of storage in the barrel (4-ethylphenol, 42.3 mg/l; 4-ethylguaiacol, 8.4 mg/l), but values increase after 15 months (4-ethylphenol, 275 mg/l; 4-ethylguaiacol, 55 mg/l) [89].

Indeed, when breeding in oak, 4-ethylphenol and 4-ethylguaiacol compounds are mainly present, but are not directly provided by oak [90]. If the presence of these molecules involves contamination with *Brettanomyces* yeast, the type of used barrels can have a pronounced effect [91]. On the one hand, used wood accumulates gradually ethylphenols in its mass (especially 4-ethylphenol), while it remains without native phenols and thermodegradation derivatives of lignin [91, 92]. On the other hand, burnt wood leads to the formation of a large number of compounds, including several volatile phenols, such as guaiacol, 4-methylguaiacol, 4-ethylguaiacol, eugenol, and syringol. This composition depends on three factors: nature, age, and origin of used barrels [31, 93]. However, the quantity of vinylphenol is less affected than the quantity of ethylphenol by the phenomenon of accumulation if wine is older [90]. It also shows that the concentration of *p*-coumaric acid in wine, which has undergone maturation in wooden barrels, as well as the phenomenon of *p*-coumaric acid extraction from wood to wine, remains unchanged [94].

Biochemical and metabolic factors

Differences in rates and balance for p-coumaric acid bioconversion into 4-ethylphenol

Volatile phenol concentrations may vary in a significant way from one wine to another and may depend on the yeast strains. Molar conversions at a rate of 90 % were reported for *D. bruxellensis*, *D. anomala*, and *P. guillermondii* while other fermentative yeasts were incapable of producing 4-ethylphenol at these rates of conversion [17]. Medawar [10] noted that with *B. intermedius*, whatever the initial concentration of coumarate is, the conversion to ethylphenol is (83 ± 5) %. This yield indicates that there is no restriction enzyme, no inhibition by substrate excess (*p*-coumaric acid), or no feedback inhibition (or repression) by ethylphenol formed in the range of studied concentrations. The yield of *p*-coumaric acid bioconversion into 4-ethylphenol was shown to be variable, as reported by different studies. Indeed, according to different authors, there is no total conversion of *p*-coumaric acid into 4-ethylphenol. Explanations are often assumptions. Thus, one explanation could be that the amount of acid leaving the mass balance would be converted within the cytoplasm by other pathways than the formation of 4-ethylphenol, such as shikimic acid and its phenylpropanoidic derivatives, stilbene or phenylalanine, and its flavonoid derivatives [10, 45].

Conversion of *p*-coumaric acid to 4-ethylphenol in wine depends on both the carbon source present in the environment and the concentration of dissolved oxygen. It is very difficult to elucidate the actual participation of each of these factors on the bioreaction and on the overall balance of bioconversion [95]. *B. bruxellensis* cannot use the *p*-coumaric acid as a sole carbon source for growth. The yield of 4-ethylphenol formation, calculated according to the amount of 4-ethylphenol generated by *p*-coumaric acid added to various synthetic environments for a strain of *B. bruxellensis*, is 2.2 % if the carbon source is acetic acid, 80 % for ethanol, and up to 92.5 % if the carbon source is glucose [95].

Biomass levels and strain diversity

Literature reveals that the most influencing factor on volatile phenol production is the presence of microorganisms responsible for biosynthesis [8]. Experiments show that if the seeding rate of *Brettanomyces* population in wine is high, the maximum population level becomes greater; the duration of the stationary phase will then be shorter, leading to an increase in 4-ethylphenol concentration. Then, the levels of volatile phenols produced are proportional to the *Brettanomyces* population that has been developed [96, 97]. In contrast, Medawar [10] proved that the measured concentration of ethylphenol does not reflect the level and the biomass growth in *Brettanomyces*. On the other side, remarkable differences were observed regarding the volatile phenol production in wine and the flora of microorganisms present. The same disparity was also observed with different strains of yeast [16, 41]. Strain variations remain the most influential and obvious factor that explains the differences observed.

Different strains of *Brettanomyces* can produce different yields of volatile phenols [8, 16, 98]. The capacity of different yeasts to generate 4-ethylphenol has been investigated, using different model media containing *p*-coumaric acid in quantities greater than those normally encountered in wine, although only *D. bruxellensis* and *D. anomala* have been associated with high conversion rates [13, 14]. Recently, it has been reported that diversity among strains for volatile phenol production differs between synthetic media and wine with regard to the maximum production levels of 4-ethylphenol and 4-ethylguaiacol [99].

Differences are believed to exist in the specificity and the mechanism of action of cinnamate decarboxylase,

involving differences between species and strains in the metabolization of hydroxycinnamic acids [29].

Growth phase

Discrepancies exist in literature for the growth phase in which *Brettanomyces* produce the highest amount of ethylphenol. Indeed, Zoekcklein [100] asserts that for *B. intermedius*, the enrichment of the environment in ethylphenols takes place when growth stops, i.e., during the stationary phase, whereas Medawar [10] has shown a higher production rate of these phenolic compounds in higher exponential growth phase than during the stationary phase. Dias et al. [4] examined *p*-coumaric acid conversion in *D. bruxellensis* during wine fermentation and showed that 4-ethylphenol production occurred roughly between the mid-exponential growth phase and the beginning of the stationary phase. The production of 4-vinylphenol was detected only in the beginning of the growth phase in low amounts because it was rapidly reduced. 4-Ethylphenol production was observed only after complete fermentation of grape juices by *S. cerevisiae* [37]. For Chatonnet et al. [31], the production of 4-ethylphenol has increased since the latent phase until the end of the exponential phase, while the production of 4-vinylphenol has increased in a first phase followed then by a decrease.

Inhibition of growth and activity

It was suggested that the decarboxylase action and the production of volatile phenols are related to yeast tolerance to phenolic compounds that are toxic [101, 102]. Thus, the growth of *Brettanomyces* could be inhibited by the presence of hydroxycinnamic acids, which could explain why yeast converts *p*-coumaric acid into 4-ethylphenol [29, 103]. Similarly, this inhibitory effect varies with *Brettanomyces* strains and with the used hydroxycinnamic acid; the combination of ferulic and *p*-coumaric acid inhibited the growth of strains to a greater extent than that of single acids [104]. This is more pronounced for *D. anomala* than for *D. bruxellensis*. This was not found by Dias et al. [4], who did not observe any inhibitory effect on hydroxycinnamic acid of *Brettanomyces* yeast, but only on those of *Saccharomyces*.

Other authors have reported that no correlation exists between yeast tolerance to phenolic acids and volatile phenol productivity. Then, they explain this bioconversion by the necessity of decarboxylation of these phenols for other metabolic pathways useful for yeast growth [41]. Morata et al. [62, 105] have studied the enological use of *S. cerevisiae* (with high HcD activity), with the aim of favoring condensation between vinylphenols and anthocyanins; this reaction forms vinylphenolic pigments that improve wine stability and color and prevent the possible metabolism of vinylphenols into ethylphenols by *Dekkera/Brettanomyces*.

4-Ethylphenol production is not inhibited by a high concentration of glucose [106]. When glucose is absent in the medium, no ethylphenol formation was detected throughout fermentation (240 h) [107]. According to Chatonnet et al. [35], the fermentation of concentrations of residual sugars close to 300 mg/l is sufficient to form a quantity of ethylphenols that surpass the set sensorial perception threshold (425 µg/l). Even for sugar concentrations below 150 mg/l and even in the presence of autolysed *S. cerevisiae*, these yeasts are able to multiply and produce ethylphenols [108] [108]. It is important to know that 4-ethylphenol concentrations in wine are not correlated with the acetic acid production [95, 109].

Dissimilarity in volatile phenol contents between red and white wines

If musts contain only trace amounts of volatile phenols, wine can contain hundreds of micrograms per liter [110]. In general, vinylphenols are predominant in white wine, while red wines are richer in ethylphenols [90]. In most cases, red wine contains 4-ethylphenol more than 4-vinylphenol. The concentration of 4-ethylphenol varies significantly between red wines [48, 82, 111, 112]. Two explanations have been proposed for this finding: The first theory states that phenolic compounds of red wine that are absent in white wines inhibit the cinnamate decarboxylase of *Saccharomyces* yeast and other microorganisms present in wine, which are capable of decarboxylating *p*-coumaric acid to vinylphenol during fermentation. But, these compounds promote the functioning of cinnamate decarboxylase of the *Brettanomyces* yeast during a contamination in red wine [113]. Logically, another explanation reveals that white wine generally contains less microorganisms, which makes the decarboxylation of *p*-coumaric acid and the reduction of 4-vinylphenol very low. If they exist in white wine, 4-vinylphenols come from winemaking. In contrast, the presence of microorganisms in red wine is frequent, as well as the presence of *Brettanomyces* yeast and the appearance of ethylphenols.

Loureiro and Malfeito-Ferreira [14] noted that the absence of high ethylphenol levels in white wine is largely ascribed to the efficiency of sulfur dioxide (SO_2) at lower pH conditions. Finally, ethylphenol formation is favored in wine at low alcohol content, because high concentration of ethanol reduces microbial activity, which delays the synthesis of ethylphenol. This production is inhibited by 13 % ethanol [95].

Hydroxycinnamate decarboxylase (HcD) and vinylphenol reductase (VPR) activities of Brettanomyces and influence of some factors

Edlin et al. [114] have purified an HcD from *B. anomalus* which was able to decarboxylate *para*-coumaric and ferulic acids, but was inactive against *ortho*-coumaric, *meta*-coumaric, and cinnamic acids, indicating that the *p*-OH is essential for its activity. Substituting the *p*-OH with a methoxy-group also inhibited the activity. The enzyme activity was shown to be constitutive and substrate inducible (i.e., the enzyme activity increases when cells are grown in the presence of increasing substrate concentrations). So, the cinnamate decarboxylase activity was induced by the presence of *p*-coumaric acid as well as 4-vinylphenol in the culture media, while VPR activity was only induced by the presence of 4-vinylphenol [2]. Moreover, the decarboxylase of *Brettanomyces/Dekkera* spp. is not inhibited by polyphenolic compounds of red wine (procyanidins and catechins) while these compounds do inhibit the decarboxylase of *S. cerevisiae* [115]. Barankowski et al. [101] showed that hydroxycinnamic acids are considered as antimicrobial agents and the action of HcD detoxifies these compounds as a stress response [116]. This function is not well understood and may have implications on the control of *Dekkera and Brettanomyces* yeasts [32]. The optimum activity of the HcD enzyme of *B. bruxellensis* was at pH 6.0 and at a temperature of 40 °C [117]; similar conditions were shown for the hydroxycinnamate decarboxylase enzyme from *B. anomalus* [114], and the highest activity was also observed in the late log phase of growth. Because it is most likely that both enzymes (HcD and VPR) belong to the same pathway, it is not surprising that they are constitutively expressed and that the highest activity is observed in the same culture phase [118] when cells reach the late phase of growth. Optimal pH for VPR is also 5–6, and when temperatures are above 50 °C or lower than 10 °C, the activity of both enzymes declines significantly. Dias et al. [95] reported that the production rate of 4-vinylphenol and 4-ethylphenol is improved by increasing temperature from 16 to 30 °C; the yield remains the same. VPR is known to be NADH dependent as the presence of NADH in the assay medium led to a 50-fold increase in the specific activity. Tchobanov [119] proposed that the enzyme, which ensures the regeneration of NAD^+, eliminates the inhibition of the alcoholic fermentation, explained by the Custer effect. Depending on strain varieties and on genetic differences, enzyme activities may act differently. Godoy et al. [117] showed that all the 12 isolates of *D. bruxellensis* tested in the study possessed HcD activity, while VPR activity does not seem to be common among them. Both enzymatic activities increased when *p*-coumaric acid was present in the culture media. The isolates that presented VPR activity had variable HcD activity, suggesting that they are not closely related, i.e., those isolates that have high HcD activity also have high VPR activity and vice versa.

Ethanol has an important impact on both enzyme activities. After few minutes of incubation in the presence of 10 or 12 % ethanol (v/v), HcD activity drastically decreases and is completely lost after 1 h, while VPR activity loses 80 and 88 % in the presence of, respectively, 10 and 12 % ethanol (v/v) [2]. Benito et al. [107] showed that when ethanol concentration exceeds 15 % (v/v), *D. bruxellensis* was unable to convert *p*-coumaric acid into 4-ethylphenol, so there was no HcD and/or VPR activity while low (5–10 %)—not very low (0 %)—ethanol concentrations favor the necessary enzymatic processes of decarboxylation and/or reduction. This might be related to the capacity of *Dekkera/Brettanomyces* to use ethanol as a carbon source.

Summary of methods used for controlling and treating the presence of *Brettanomyces/Dekkera* and the production of ethylphenols in wine

The quantity of volatile phenols produced in wine depends directly on the hydroxycinnamic acid composition, the contamination by microorganisms, and the accumulation of formed volatile compounds. Several recent researches are under study to stop or prevent wine contamination by the yeast *Brettanomyces*.

Chemical methods

The mainly used preservative is sulfur dioxide which is effective in both prefermentation and post-fermentation stages. A concentration of 30 mg/l of free SO_2 is required to be effective against these yeast activities [113]. Some authors observed yeast growth with concentration of free SO_2 above 30 mg/l, reflecting certain *D. bruxellensis* strains resistance [120]. This controversy does not lie in the free form of SO_2, but rather in the actual effectiveness of its molecular form [121], which is dependent on many variations in wine composition (pH, ethanol, temperature, anthocyanin levels, and nutrient content) [36, 122]. It is important to note that oxygen availability influences the effectiveness of molecular SO_2 on a strain of *B. bruxellensis*. The strain can remain viable and proliferate in contact with oxygen [21].

Recently, winemakers use less SO_2 to alleviate concerns about health implications since it has been shown that SO_2 is related to pseudo-allergies. Izquierdo-Cañas et al. [123] studied the effectiveness of a colloidal silver complex (CSC) (silver nanoparticles) as an alternative antiseptic to SO_2 in both white and red winemaking. CSC at the doses of

1 g/kg of grape was able to control acetic acid and lactic acid bacteria development, without affecting the growth of *S. cerevisiae*. Silver concentration in finished white and red wine was well below legal limits, but CSC wine had lower values of alcoholic degree and acetaldehyde content than SO_2 wine, but no differences in aroma and taste were detectable between both wines.

The insoluble polyvinylpolypyrrolidone (PVPP) was commercially introduced in 1961 as an adsorbent for phenols of beer and wine [124]. PVPP is a synthetic clarification material with high molecular weight composed of cross-linked monomers of polyvinylpyrrolidone with affinity for low molecular weight phenolics, such as *p*-coumaric acid.

The mechanism of action is hydrogen bond formation between carbonyl groups on the polyamide (PVPP) and the phenolic hydrogen [125, 126]. Being a selective phenol absorbent, PVPP exists in various particle sizes, and the legal limit authorized by the EU regulation is 80 g/hl. In general, it is used at rates between 12 and 72 g/hl. The use of PVPP generally induces a decrease in total polyphenols, phenolic acids, hydroxycinnamic acids, procyanidins, catechins and polyphenols and protein complexes. Results obtained by Lisanti et al. [127] revealed that a treatment with PVPP leads to a 11 % decrease in the concentration of 4-ethylphenol in low contaminated red wine, while at higher concentrations (1970 μg/l of 4-ethylphenol and 126 μg/l of 4-ethylguaiacol), the decrease in volatile phenol levels was not statistically significant for all fining agents. The effectiveness of PVPP is extensively discussed in literature and is even contradictory [127]. It is also worth mentioning that the effect of clarifying agents is not the same for the elimination of a compound, where the treatment material is added in pre- or post-fermentative phase.

It is interesting to note that very few studies show effectiveness of chemical treatments (ozonation, antiseptics, dimethyldicarbonate (DMDC) commercially known as Velcorin, etc.) specifically against *Brettanomyces*.

Ozone was shown to be a highly effective sanitizing agent without interfering with the profile of the phenolic substances extracted from oak. The effectiveness of ozone in eliminating microorganisms was evaluated in aqueous solution at several cell and ozone concentrations. At a cell concentration of less than 10^3 CFU/ml, ozone was able to eliminate wine spoilage microorganisms. At a high cell concentration, the presence of organic matter reduced the effectiveness of ozone [128]. Cantacuzene et al. [129] found a reduction in *Brettanomyces* population with ozone gas and with hot water treatment, but not with aqueous ozone, while Coggan [130] noted the reduction of 99 % of *Brettanomyces* population with ozonated water. Renouf et al. [131] advised the use of DMDC after the end of malolactic fermentation as it can act on fermenting species, such as *S. cerevisiae* and *Oenococcus oeni*, and recommended its use after bottling. Costa et al. [132] suggested that, for an initial inoculum of 500 CFU/ml, the minimum inhibitory concentration (MIC) of yeast species *Schizosaccharomyces pombe*, *D. bruxellensis*, *S. cerevisiae*, and *P. guilliermondii* was 100 mg/l. They also noted that *Zygosaccharomyces bailii*, *Zygoascus hellenicus*, and *Lachancea thermotolerans* are the most sensitive strains, with a MIC of 25 mg/l DMDC. When inoculation rates increase to 10^6 CFU/ml, the maximum dose of DMDC legally authorized (200 mg/l) was not effective against the most resistant species. The addition of 100 mg/l potassium metabisulfite, equivalent to 1 mg/l molecular sulfur dioxide, increased the inactivation effect of 100 mg/l DMDC over initial yeast populations of 10^6 CFU/ml, but did not fully kill *S. pombe* and *S. cerevisiae*.

It has been reported that the use of 3–6 g/l of a polysaccharide derived from chitin, called chitosan, drastically decreases the growth of *B. bruxellensis* and *B. intermedius* in mixed bioethanol fermentation with *S. cerevisiae* [133]. So far, the reaction mechanism of chitosan against *Brettanomyces* has not been well understood. The antimicrobial activity of chitosan is effective from 5 to 10 days on different strains of *B. bruxellensis* while *S. cerevisiae,* alcohol, and malolactic fermentation were not affected. However, it is recommended to inoculate lactic acid bacteria 8 days after the chitosan treatment [134]. "No Brett inside"—known as a natural polysaccharide extracted from a fungal source of chitin (*Aspergillus niger*)—is an effective preventive tool against *Brettanomyces*, which is commercialized by "Lallemand" company. It was accepted as a new practice by enological codex in July 2009 by the Organisation Internationale de la Vigne et du Vin (OIV) and has been allowed by the European Union since December 2010. The recommended dosage is 4 g/hl, while the maximum authorized concentration is 10 g/hl.

The ability of four polymers (cellulose acetate, cellulose acetate propionate (CAP), cellulose acetate butyrate, and cellulose propionate (CP) fibers) to reduce 4-ethylphenol and 4-ethylguaiacol was recently evaluated in wine. CAP and CP performed best results, but CAP was the most promising because of its more favorable Food and Drug Administration (FDA) classification for food contact substances. Doses of up to 20 g/l of CAP and wine contact times of up to 60 min were tested. Using 4 g/l, an average of 31–32 % of both phenols was reduced in defective red wines. This wine treatment affected neither color nor total proanthocyanidins and catechins, and wines were judged to be better than the corresponding spoiled controls. The advantage of CAP fiber is that it can be regenerated by washing with ethanol or aqueous solution (pH 12), without remarkable changes in depletion efficiency [135].

Biological methods

Recently, biological control procedures have been considered as desirable alternatives to chemical ones [136]. Several studies are being developed to avoid yield losses caused by *Brettanomyces* in the wine industry. An alternative approach, based on antimicrobial peptides derived from a natural protein, was described by Enrique et al. [137]. Results showed that lactoferrin-derived peptides, either LfcinB17-31 or a pepsin LF hydrolysate, inhibit the growth of *D. bruxellensis* when growing in laboratory medium and wine. Moreover, the efficiency of LfcinB17-31 depends on *D. bruxellensis* strains and on the food matrix.

Benito et al. [138] proposed a new biological strategy to minimize ethylphenol precursors in red wine by the formation of pyroanthocyanins from S. cerevisiae strains with high HcD activity. This activity significantly increased the formation of vinylphenolic pyranoanthocyanins and reduced the final concentration of 4-ethylphenol and 4-ethylguaiacol generated by the VPR activity of D. bruxellensis. By using this method, ethylphenol concentrations were reduced to 70 %. Another study described a natural strategy to reduce the formation of ethylphenols in wines contaminated by *Dekkera/Brettanomyces*, which is related to the combined effect of using cinnamyl esterases and HcD+ *Saccharomyces* strains. The musts treated with cinnamyl esterases and later fermented with HcD+ yeast strains showed lower contents of 4-ethylphenol than those fermented with HcD− strains. This reduction in the ethylphenol content is due to the transformation of hydroxycinnamic acids into stable vinylphenolic pyranoanthocyanins pigments [139].

Another biocontrol agent, which is very suitable for winemaking applications, has been described by Santos et al. [140]. It is a new killer toxin (PMKT2) produced by *P. membranifaciens* that has both activity and stability compatible with pH and temperature values of wine production. PMKT2 showed killer activity against *B. bruxellensis* in similar conditions as those prevailing in wine fermentation, while *S. cerevisiae* was fully resistant, indicating that PMKT2 is compatible with the fermentative process. Moreover, Santos et al. [136] proved the killer activity of *Ustilago maydis* to be effective against *B. bruxellensis* as this strain is capable of producing a KP6-related toxin at pH values between 3.0 and 4.5 and temperatures between 15 and 25 °C while *S. cerevisiae* is fully resistant to its killer activity. In addition to the observed growth inhibition, small amounts of the toxin are able to reduce the production of responsible volatile phenols, thus controlling the aroma defects in wine caused by *B. bruxellensis*.

Among the various types of materials used in the clarification, yeast lees have a direct effect on the composition of volatile phenols in wine. Indeed, studies of Chassagne et al. [141] reveal that ethylphenols can be eliminated from the environment by adsorption on yeast lees. These lees are mainly composed of produced and dead yeast, mixed with tartaric salts, bacteria, and cell debris. They are used in breeding wine and actively involved in the adsorption of these phenolic compounds. An interaction happens between wine polyphenols and proteins by linking van der Waals bonds [121, 142, 143]. Although this interaction is limited, it has a pronounced effect on the phenolic composition of wine [144]. It seems that there is a correlation between the adsorption of polyphenols and the presence of live or dead yeast in wine [145]. Depending on the varieties of yeasts and depending on the environment, the relative effectiveness of adsorption differs.

The use of yeast hulls presents an eco-friendly alternative to conventional physicochemical techniques used to decrease volatile phenols in wine. *S. cerevisiae* mutant strains with deletion of genes encoding specific proteins involved in cell wall structure and composition were studied, and a major role of mannoprotein (occurring in the cell wall or hull) in 4-ethylphenol sorption was identified. The sorption capacity of 4-ethylphenol by yeasts was greatly influenced by the strain nature, the methods and medium used for biomass production, and the drying of yeasts after harvesting as well. It was confirmed that 4-ethylphenol sorption occurs at the surface of the yeast wall and that not all mannoproteins are determinants of sorption; the sorption capacity of cells with deletion of Gas1*p*-encoding gene was 75 % lower than that of wild type.

Physical methods

Physical treatments (flash pasteurization, sterilization, etc.) do not seem to be as effective as sulfitation [16]. The cross-flow filtration is an alternative curative technology, generally used for microbiological stabilization where the risk of alteration is important. It can reduce the microflora populations by 1000-fold, but yeast in a viable-but-non-cultivable (VBNC) state is small in size and may pass through the 0.45-μm filtration membrane [12] as *Brettanomyces* is believed to possibly reduce their size when entering the VBNC state. Suárez et al. [17] reported that sterile filtration or the fining proteins are effective against any type of microorganisms, while Couto et al. [146] noted that these procedures are not totally effective so they can negatively affect the sensorial properties of wine [147]. Latest findings suggest that successful removal by filtration was a strain-specific characteristic. Authors suggested that the addition of 0.5 mg/l of molecular SO_2 in combination with 1.2 μm membrane filtration decreased populations of all spoilage strains tested to obtain a microbiologically stable wine [148].

A method for removing volatile phenols by *Brettanomyces* was developed and validated at Inter Rhone. The treatment process is based on a membrane technology coupled with an adsorbent. This matrix adsorption specifically eliminates some of the volatile phenols after an assembly of the wine treated with the one untreated. Indeed, it reduces the concentration of 90 % of volatile phenols while most of the enological standard analyses showed no change, except in the alcoholic degree that decreases by 5 % [149]. Another technique to reduce 4-ethylphenol concentrations was developed by Palomero et al. [150], who reported the capacity of lyophilized *S. cerevisiae* G37 and *Schizosaccharomyces pombe* 936 to adsorb 4-ethylphenol. The impact of this treatment was significant reductions in the wine anthocyanin content, leading to color loss when the yeast dose increased, so the problem is whether this technique could be of practical use.

A new system using pulsed electric fields technology (PEF) has been developed by Puértolas et al. [151] to control microbiological contamination in wineries, especially *Dekkera* and *Lactobacillus*. They established an optimum treatment which permitted to reduce 99.9 % of the spoilage flora of must and wine. After applying PEF treatments at the most intensive intensities, no changes in color and odors were observed. In general, all microbial species were more sensitive in wine than in must. Only *Lactobacillus plantarum* revealed higher PEF resistance in wine. The different behavior concerning PEF resistance in must and wine could be attributed to high ethanol concentration in wine (13 % v/v). Among yeasts, *D. bruxellensis* in must and *S. bayanus* in wine were the most sensitive microorganisms, and *D. anomala* was the most PEF resistant, independently from the medium.

In addition, the effective use of low electric current treatment (LEC) in the winemaking process can prevent the growth of undesirable *Dekkera* yeasts [152]. The LEC treatment caused alterations in the morphology and integrity of cells. When comparing with the control that did not submit a treatment, rupturing of the membrane system with a loss of cell organization was observed while no evident changes in color, odor, or other criteria (floral, reduced, acetic) were mentioned on the 60th day in wine before and after LEC treatments at 200 mA intensities.

Combining high-power ultrasonics (HPU) with hot water of at least 60 °C was also able to eliminate cultivable *D. bruxellensis* inoculated on the surface (0–2 mm) and up to 4 mm into the oak itself. Wine stored in HPU-treated barrels did not show any differences in their extraction of oak compounds, nor could these wines be differentiated by a sensory panel. Thus, HPU did not adversely affect oak extraction into wine [153].

The effect of high hydrostatic pressure (HHP), which can be regarded as a form of cold pasteurization, on wines contaminated with *Dekkera/Brettanomyces* populations of 10^4 and 10^6 CFU/ml growing at either pH 3.2 or 3.6 and at room temperature (25 °C) was studied. The HHP treatment (100 MPa for 24 h) was highly effective at controlling the growth of all combinations of starting yeast population and pH, without causing significant modifications of thermosensitive wine molecules such as pigments and volatile compounds [154].

Recently, the effectiveness of microwave technology which is a short treatment (1 min repeated 3 times) based on the use of a pulse train generator of high frequency (3,000 W) was tested in oak barrels, in order to remove microorganisms in depth (8 mm) of inside of the barrel staves. The microwave treatment did not affect chemical wood quality despite having effects in the microbial populations; from 35 to 67 % of the *B. bruxellenxis* population in French and American oak, respectively, were eliminated [155]. The percentage of microorganism reduction was greater in American oak staves due to the different uses of the barrels and also to the higher porosity of the French oak wood, which would favor a higher wine penetration, increasing the difficulty in cleaning and sanitizing them [156].

Conclusion

Volatile phenols such as 4-ethylphenol, 4-ethylguaiacol, 4-vinylphenol, and 4-vinylguaiacol are considered to be primarily responsible for "off-flavors" and induce significant problems that arise in winemaking. *Brettanomyces* is the wild yeast implicated in this spoilage and has long been associated with these volatile phenols as analysis of wine for these compounds enables wineries to identify the presence of *Brettanomyces* populations large enough to impact wine sensory effects. This yeast genus is difficult to be eliminated once established in a winery. This review gave a comprehensive study based on the levels and factors governing the production of undesirable metabolites. It also shows methods cited in literature, for monitoring the risk from *Brettanomyces*, making the winemaking process control easier.

Conflict of interest Joyce Kheir has received research grants from "Le Conseil de la Recherche de l'Universite Saint-Joseph, Beirut, Lebanon". Dominique Salameh, Pierre Streihaiano, Cedric Brandam and Roger Lteif declare that they have no conflict of interest.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

References

1. Fleet GH (1993) The microorganisms of winemaking-isolation enumeration and identification. In: Fleet GH (ed) Wine Microbiology and Biotechnology. Harwood Academic Publishers, Chur, pp 1–27

2. Godoy L, Martínez C, Carrasco N, Ganga MA (2008) Purification and characterization of a p-coumarate decarboxylase and a vinylphenol reductase from Brettanomyces bruxellensis. Int J Food Microbiol 127:6–11
3. Rodrigues N, Gonçalves G, Pereira-da-Silva S, Malfeito-Ferreira M, Loureiro V (2001) Development and use of a new medium to detect yeasts of the genera Dekkera/Brettanomyces. J Appl Microbiol 90:588–599
4. Dias L, Dias S, Sancho T, Stender H, Querol A, Malfeito-Ferreira M, Loureiro V (2003) Identification of yeasts isolated from wine-related environments and capable of producing 4-ethylphenol. Food Microbiol 20:567–574
5. Caboni P, Sarais G, Cabras M, Angioni A (2007) Determination of 4-ethylphenol and 4-ethylguaiacol in wines by LC-MS-MS and HPLC-DAD-Fluorescence. J Agr Food Chem 55:7288–7293
6. Chatonnet P, Boidron JN, Pons M (1990) Elevage des vins rouges en fûts de chêne: évolution de certains composés volatils et de leur impact arômatique. Sci Aliment 10:565–587
7. Snowdon EM, Bowyer MC, Grbin PR, Bowyer PK (2006) Mousy Off-Flavor: a Review. J Agr Food Chem 54:6465–6474
8. Silva P, Cardoso H, Geros H (2004) Studies on the wine spoilage capacity of Brettanomyces/Dekkera spp. Am J Enol Vitic 55:65–72
9. Van Nedervelde L, Debourg A (1995) Properties of Belgian acid beers and their microflora - Part 2: biochemical properties of Brettanomyces yeasts. Cerevesia 20:43–48
10. Medawar W (2003) Etude physiologique et cinétique des levures du genre Brettanomyces dans un contexte oenologique. Thèse de doctorat de l'Institut national polytechnique de Toulouse, France et de l'Université Saint Joseph de Beyrouth, Liban
11. Aguilar Uscanga MG, Delia ML, Strehaiano P (2000) Nutritional requirements of Brettanomyces bruxellensis: growth and physiology in batch and chemostat cultures. Canadian J Microbiol 46:1046–1050
12. Millet V, Lonvaud-Funel A (2000) The viable but non-culturable state of wine micro-organisms during storage. Lett Appl Microbiol 30:136–141
13. Benito S, Palomero F, Morata A, Calderón F, Suárez-Lepe JA (2009) A method for estimating Dekkera/Brettanomyces populations in wines. J Appl Microbiol 106:1743–1751
14. Loureiro V, Malfeito-Ferreira M (2006) Spoilage activities of Dekkera/Brettanomyces spp. In: Blackburn CW (ed) Food spoilage microorganisms. Chapter 13. Woodhead Publishers, Cambridge, pp 354-398
15. Conterno L, Joseph CML, Arvik TJ, Henick-Kling T, Bisson LF (2006) Genetic and physiological characterization of brettanomyces bruxellensis strains isolated from wines. Am J Enol Vitic 57:139–147
16. Barbin P (2006) Contrôle et éléments de la contamination par la levure Brettanomyces au cours du procédé de vinification en rouge. Thèse de doctorat de l'Institut national polytechnique de Toulouse, France
17. Suárez R, Suárez-Lepe JA, Morata A, Calderón F (2007) The production of ethylphenols in wine by yeasts of the genera Brettanomyces and Dekkera: a review. Food Chem 102:10–21
18. Renouf V, Lonvaud-Funel A (2006) Development of an enrichment medium to detect Dekkera/Brettanomyces bruxellensis, a spoilage wine yeast, on the surface of the grape berries. Microbiol Res 162:154–167
19. Ciani M, Maccarelli F, Fatichenti F (2003) Growth and fermentation behaviour of Brettanomyces/Dekkera yeasts under different conditions of aerobiosis. World J Microb Biot 19:419–422
20. Ciani M, Ferraro L (1997) Role of oxygen on acetic acid production by Brettanomyces/Dekkera in winemaking. J Sci Food Agr 75:489–495
21. Du Toit WJ, Pretorius IS, Lonvaud-Funel A (2005) The effect of sulphur dioxide and oxygen on the viability and culturability of a strain of Acetobacter pasteurianus and a strain of Brettanomyces bruxellensis isolated from wine. J Appl Microbiol 98:862–871
22. Barata A, Pagliara D, Piccininno T, Tarantino F, Ciardulli W, Malfeito-Ferreira M, Loureiro V (2008) The effect of sugar concentration and temperature on growth and volatile phenol production by Dekkera bruxellensis in wine. FEMS Yeast Res 8:1097–1102
23. Barata A, Caldeira J, Botelheiro R, Pagliara D, Malfeito-Ferreira M, Loureiro V (2008) Survival patterns of Dekkera bruxellensis in wines and inhibitory effect of sulphur dioxide. Int J Food Microbiol 121:7
24. Jin ZM, He JJ, Bi HQ, Cui XY, Duan CQ (2009) Phenolic compound profiles in berry skins from nine red wine grape cultivars in Northwest China. Molecules 14:4922–4935
25. Castillo-Sánchez JX, García-Falcón MS, Garrido J, Martínez-Carballo E, Martins-Dias LR, Mejuto XC (2008) Phenolic compounds and colour stability of Vinhão wines: influence of wine-making protocol and fining agents. Food Chem 106:18–26
26. Basha SM, Musingo M, Colova VS (2004) Compositional differences in the phenolics compounds of muscadine and bunch grape wines. Afr J Biotechnol 3:523–528
27. Rentzsch M, Wilkens A, Winterhalter P (2009) Non-flavonoid phenolic compounds. In: Moreno-Arribas MV, Polo C (eds) Wine chemistry and biochemistry. Springer, New York, pp 509–527
28. Gerbaux V, Vincent B, Bertrand A (2002) Influence of maceration temperature and enzymes on the content of volatile phenols in Pinot noir wines. Am J Enol Vitic 53:131–137
29. Edlin DAN, Narbad A, Dickinson JR, Lloyd D (1995) The biotransformation of simple phenolic compounds by Brettanomyces anomalus. FEMS Microbiol Lett 125:311–315
30. Heresztyn T (1986) Metabolism of volatile phenolic compounds from hydroxycinnamic acids by Brettanomyces yeast. Arch Microbiol 146:96–98
31. Chatonnet P, Dubourdieu D, Boidron JN, Pons M (1992) The origin of ethylphenols in wines. J Sci Food Agr 60:165–178
32. Harris V, Ford C, Jiranek V, Grbin P (2009) Survey of enzyme activity responsible for phenolic off-flavour production by Dekkera and Brettanomyces yeast. Appl Microbiol Biot 81:1117–1127
33. Edlin D (1997) The production of phenolic flavour compounds by yeasts and fungi. PhD thesis, University of Cardiff, United Kingdom
34. K O'Toole D, Kun Lee Y (2006) Food involving yeast and ethanol fermentation. In: Kun Lee Y (ed) Microbial biotechnology: principles and applications. Chapter 9. World Scientific Publishing Co. Pte. Ltd, Singapore
35. Chatonnet P, Dubourdieu D, Boidron JN (1995) The influence of Brettanomyces/Dekkera sp. yeasts and lactic acid bacteria on the ethylphenol content of red wines. Am J Enol Vitic 46:463–468
36. Oelofse A, Pretorius IS, Du Toit M (2008) Significance of Brettanomyces and Dekkera during winemaking: a synoptic review. S Afr J Enol Vitic 29:128–144
37. Vanbeneden N (2007) Release of hydroxycinnamic acids and formation of flavour-active volatile phenols during the beer production process. PhD thesis, Katholieke Universiteit Leuven, Belgium
38. Barata A, Nobre A, Correia P, Malfeito-Ferreira M, Loureiro V (2006) Growth and 4-ethylphenol production by the yeast Pichia guilliermondii in grape juices. Am J Enol Vitic 57:133–138
39. Gerbaux V, Briffox C, Vincent B (2003) Optimisation de la macération finale à chaud, intérêt d'un enzymage et d'une macération sous chapeau immergé pour la vinification du pinot noir. Revue Française d'Œnologie 201:16–21

40. Fugelsang KC, Zoecklein BW (2003) Population dynamics and effects of Brettanomyces bruxellensis strains on Pinot noir (Vitis vinifera L.) wines. Am J Enol Vitic 54:294–300
41. Shinohara T, Kubodera S, Yanagida F (2000) Distribution of phenolic yeasts and production of phenolic off-flavors in wine fermentation. J Biosci Bioeng 90:90–97
42. Ho P, Hogg TA, Silva MCM (1999) Application of a liquid chromatographic method for the determination of phenolic compounds and furans in fortified wines. Food Chem 64:115–122
43. Avar P, Nikfardjam MSP, Kusagi-Mate S, Montsko G, Szabo Z, Boddi K, Ohmacht R, Mark L (2007) Investigation of phenolic components of Hungarian wines. Int J Mol Sci 8:1028–1038
44. Pöllnitz AP, Pardon KH, Sefton MA (2000) Quantitative analysis of 4-ethylphenol and 4-ethylguaiacol in red wine. J Chromatogr A 874:101–109
45. Goldberg DM, Tsang E, Karumanchiri A, Soleas GJ (1998) Quercetin and p-coumaric acid concentrations in commercial wines. Am J Enol Vitic 49:142–151
46. Asselin C, Pages J, Morlat R (1987) Résultats de 3 années d'étude concernant la mise en évidence de l'effet terroir sur les caractéristiques des vins à l'aide de l'analyse sensorielle. 3ème symposium international sur la physiologie de la vigne. Bulletin de l'OIV, pp 316-323
47. Falcetti M, Asselin C (1996) Les effets du terroir ou l'expression des potentiels à valoriser. In: Asselin C MJ, Sapis JC, Flanzy C (eds) 1er Colloque international « Les terroirs viticoles », URVV Angers, ISVV Montpellier, pp 253-258
48. Ducruet V, Flanzy C, Bourzeix M, Chambroy Y (1983) Les constituants volatils des vins jeunes de macération carbonique. Sci Aliment 3:413–426
49. Etievant PX, Issanchou SN, Marie S, Ducruet V, Flanzy C (1989) Sensory impact of volatile phenols on red wines aroma: influence of carbonic maceration and time of storage. Sci Aliment 9:19–33
50. Goldsworthy SA (1993) Pre-fermentation maceration of Pinot noir wine. M Applied Science Thesis. Lincoln University, New Zealand
51. Heatherbell D, Dicey M, Goldsworthy S, Vanhanen L (1997) Effect of prefermentation cold maceration on the composition, color and flavor of Pinot noir wine. In: Henick-Kling T (ed) Proceedings of the 4th international symposium on cool climate enology and viticulture. USA, New York, pp 10–17
52. Koyoma K, Goto-Yamamoto N, Hashizume K (2007) Influence of maceration temperature in red wine vinification on extraction of phenolics from berry skins and seeds of grape (Vitis vinifera). Biosci Biotech Bioch 71:958–967
53. Gerbaux V (1993) Etude de quelques conditions de cuvaison susceptible d'augmenter la composition polyphénolique des vins de Pinot noir. Revue des Œnologues 69:15–18
54. Nagel CW, Baranowski JD, Wulf LW, Powers JR (1979) The hydroxycinnamic acid tartaric acid ester content of musts and grape varieties grown in the Pacific Northwest. Am J Enol Vitic 30:198–201
55. Salameh D, Brandam C, Medawar W, Lteif R, Strehaiano P (2008) Highlight on the problems generated by p-coumaric acid analysis in wine fermentations. Food Chem 107:1661–1667
56. Cabrita MJ, Palma V, Patao R, Freitas MC (2012) Conversion of hydroxycinnamic acids into volatile phenols in a synthetic medium and in red wine by Dekkera bruxellensis. Ciênc Tecnol Aliment 32:106–111
57. Sacchi KL, Bisson LF, Adams DO (2005) A review of the effect of winemaking techniques on phenolic extraction in red wines. Am J Enol Vitic 56:197–206
58. Vasserot Y, Caillet S, Maujean A (1997) Study of anthocyanin adsorption by yeast lees. Effect of some physicochemical parameters. Am J Enol Vitic 48:433–437
59. Delcroix A, Gunata Z, Sapis J-C, Salmon J-M, Bayonove C (1994) Glycosidase activities of three enological yeast strains during winemaking: effect on the terpenol content of Muscat wine. Am J Enol Vitic 45:291–296
60. Sponholz WR (1997) L'attività enzimatica dei livetti e la stabilità del colore rosso dei vini. Vigne vini 24:34–36
61. Monagas M, Nunez V, Bartolome B, Gomez-Cordoves C (2003) Anthocyanin-derived pigments in Graciano, Tempranillo, and Cabernet Sauvignon wines produced in Spain. Am J Enol Vitic 54:163–169
62. Morata A, Gómez-Cordovés MC, Colomo B, Suárez JA (2005) Cell wall anthocyanin adsorption by different Saccharomyces strains during the fermentation of Vitis vinifera L. cv Graciano grapes. Eur Food Res Technol 220:341–346
63. Dallas C, Ricardo-da-Silva JM, Laureano O (1996) Interactions of oligomeric procyanidins in model wine solutions containing malvidin-3-glucoside and acetaldehyde. J Sci Food Agr 70:493–500
64. Morata A, Gómez-Cordovés MC, Colomo B, Suárez JA (2003) Pyruvic acid and acetaldehyde production by different strains of Saccharomyces cerevisiae: relationship with Vitisin A and B formation in red wines. J Agr Food Chem 51:7402–7409
65. Bakker J, Timberlake CF (1997) Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. J Agr Food Chem 45:35–43
66. Vivar-Quintana AM, Santos-Buelga C, Rivas-Gonzalo JC (2002) Anthocyanin-derived pigments and colour of red wines. Anal Chim Acta 458:147–155
67. Gómez-Plaza E, Gil-Muñoz R, López-Roca JM, De la Hera-Orts ML, Martínez-Cultíllas A (2000) Effect of the addition of bentonite and polyvinylpolypyrrolidone on the colour and long-term stability of red wines. J Wine Res 11:223–231
68. Francis FJ (1992) A new group of food colorants. Trends Food Sci Tech 3:27–30
69. Liao H, Cai Y, Haslam E (1992) Polyphenol interactions. Anthocyanins: co-pigmentation and colour changes in red wines. J Sci Food Agr 59:299–305
70. Baublis A, Spomer ART, Berber-Jiménez MD (1994) Anthocyanin pigments: comparison of extract stability. J Food Sci 59:1219–1221
71. Dugelay I, Baumes R, Gunata Z, Razungles A, Bayonove C (1995) Evolution de l'arôme au cours de la conservation du vin : formation de 4-(1-éthoxyéthyl)-phénol et 4-(1-éthoxyéthyl)-gaïacol. Sci Aliment 15:423–433
72. Eskin NAM (1990) Biochemistry of food processing: browning reactions in foods. In: Eskin NAM (ed) Biochemistry of Foods. Academic Press, London
73. Macheix JJ, Sapis JC, Fleuriet A, Lee CY (1991) Phenolic compounds and polyphenoloxidase in relation to browning in grapes and wines. Crit Rev Food Sci 30:441–486
74. Singleton VL (1987) Oxygen with phenols and related reactions in musts, wines, and model systems: observations and practical implications. Am J Enol Vitic 38:69–77
75. Cheynier V, Fulcrand H (1998) Oxydation des polyphénols dans le moût et les vins. In: Flanzy C (ed) Œnologie-fondements scientifiques et technologiques. Technique et Documentation, Lavoisier, pp 580–594
76. Giusti MMn, Rodríguez-Saona LE, Wrolstad RE (1999) Molar absorptivity and color characteristics of acylated and non-acylated pelargonidin-based anthocyanins. J Agr Food Chem 47:4631–4637
77. Mayer AM (1986) Polyphenol oxidases in plants-recent progress. Phytochemistry 26:11–20
78. Okuda T, Kimura Y, Yoshida T, Hatano T, Okuda H, Arichi S (1983) Studies on the activities of tannins and related compounds from medicinal plants and drugs. I. Inhibitory effects on

lipid peroxidation in mitochondria and microsomes of liver. Chem Pharm Bull 31:1625–1631
79. Guyot S, Vercauteren J, Cheynier V (1996) Structural determination of colourless and yellow dimers resulting from (+)-catechin coupling catalysed by grape polyphenoloxidase. Phytochemistry 42:1279–1288
80. Dugelay I, Gunata Z, Sapis JC, Baumes R, Bayonove C (1993) Role of cinnamoyl esterase activities from enzyme preparations on the formation of volatile phenols during winemaking. J Agr Food Chem 41:2092–2096
81. Rayne S, Eggers N (2007) Volatile phenols from Brettanomyces in barreled red wines: are they stable? And where do they reside? Aust NZ Grapegrow Winemak 522:64–69
82. Di Stefano R (1985) Gli etil fenoli nei vini. Vigne vini 5:35–38
83. Dubois P, Dekimpe J (1982) Constituants volatils odorants des vins de Bourgogne élevés en fûts de chêne neufs. Revue Française d'Œnologie 88:51–53
84. Dubois P (1989) Apports du fut de chêne neuf à l'arôme des vins. Revue Française d'Œnologie 120:19–24
85. Kadim D, Mannheim CH (1999) Kinetics of phenolic extraction during aging of model wine solution and white wine in oak barrels. Am J Enol Vitic 50:33–39
86. Garde-Cerdán T, Ancín-Azpilicueta C (2006) Effect of oak barrel type on the volatile composition of wine: storage time optimization. LWT Food Sci Technol 39:199–205
87. Vivas N, Glories Y (1993) Les phénomènes d'oxydo-réduction liés à l'élevage en barrique des vins rouges: aspect technologique. Revue Française d'Œnologie 142:33–38
88. Peyron D, Boukhrta M, Feuillat M (1994) Evolution de la composition phénolique des vins rouges en relation avec la qualité des bois de chêne de tonnellerie. Revue Française d'Œnologie 146:5–10
89. Garde-Cerdán T, Ancín-Azpilicueta C (2006) Review of quality factors on wine ageing in oak barrels. Trends Food Sci Tech 17:438–447
90. Boidron JN, Chatonnet P, Pons M (1988) Influence du bois sur certaines substances odorantes des vins. Connaiss Vigne Vin 22:275–294
91. Garde-Cerdán T, Torrea-Goñi D, Ancín-Azpilicueta C (2004) Accumulation of volatile compounds during ageing of two red wines with different composition. J Food Eng 65:349–356
92. Pérez-Prieto LJ, López-Roca JM, Martínez-Cutillas A, Pardo-Mínguez F, Gómez-Plaza E (2003) Extraction and formation dynamic of oak-related volatile compounds from different volume barrels to wine and their behavior during bottle storage. J Agr Food Chem 51:5444–5449
93. Garde-Cerdán T, Rodríguez-Mozaz S, Ancín-Azpilicueta C (2002) Volatile composition of aged wine in used barrels of French oak and of American oak. Food Res Int 35:603–610
94. Matejícek D, Mikes O, Klejdus B, Sterbová D, Kubán V (2005) Changes in contents of phenolic compounds during maturing of barrique red wines. Food Chem 90:791–800
95. Dias L, Pereira-da-Silva S, Tavares M, Malfeito-Ferreira M, Loureiro V (2003) Factors affecting the production of 4-ethylphenol by the yeast Dekkera bruxellensis in enological conditions. Food Microbiol 20:377–384
96. Gerbaux V, Jeudy S, Monamy C (2000) Étude des phénols volatils dans les vins de Pinot noir en Bourgogne. Bulletin de l'OIV 73:581–599
97. Renouf V, Falcou M, Miot-Sertier C, Perello MC, De Revel G, Lonvaud-Funel A (2006) Interactions between Brettanomyces bruxellensis and other yeast species during the initial stages of winemaking. J Appl Microbiol 100:1208–1219
98. Valentão P, Seabra RM, Lopes G, Silva LR, Martins V, Trujillo ME, Velázquez E, Andrade PB (2007) Influence of Dekkera bruxellensis on the contents of anthocyanins, organic acids and volatile phenols of Dão red wine. Food Chem 100:64–70
99. Oelofse A, Lonvaud-Funel A, du Toit M (2009) Molecular identification of Brettanomyces bruxellensis strains isolated from red wines and volatile phenol production. Food Microbiol 26:377–385
100. Zoekcklein BW (2000) Brettanomyces aroma/flavor research. Vinter's Corner. 15(1). http://www.fst.vt.edu/Zoecklein/Janfeb00.html
101. Baranowski JD, Davidson PM, Nagel CW, Branen AL (1980) Inhibition of Saccharomyces cerevisiae by naturally occurring hydroxycinnamates. J Food Sci 45:592–594
102. Goodey AR, Tubb RS (1982) Genetic and biochemical analysis of the ability of Saccharomyces cerevisiae to decarboxylate cinnamic acids. J Gen Microbiol 128:2615–2620
103. Dubourdieu D, Darriet P, Chatonnet P (1989) Intervention of enzymatic systems of Saccharomyces cerevisiae on some precursors of grape aroma. XIIth International symposium specialized on yeast Louvain, Belgium
104. Harris V, Ford C, Jiranek V, Grbin P (2008) Dekkera and Brettanomyces growth and utilisation of hydroxycinnamic acids in synthetic media. Appl Microbiol Biot 78:997–1006
105. Morata A, Gómez-Cordovés MC, Colomo B, Suárez JA (2006) Effects of pH, temperature and SO_2 on the formation of pyranoanthocyanins during red wine fermentation with two species of Saccharomyces. Int J Food Microbiol 106:123–129
106. Malfeito-Ferreira M, Rodrigues N, Loureiro V (2001) The influence of oxygen on the ''horse sweat taint'' in red wines. Italian Food Bev Technol 24:34–38
107. Benito S, Palomero F, Morata A, Calderón F, Suárez-Lepe JA (2009) Factors affecting the hydroxycinnamate decarboxylase/vinylphenol reductase activity of Dekkera/Brettanomyces: application for Dekkera/Brettanomyces control in red wine making. J Food Sci 74:M15–M22
108. Chassagne D, Charpentier C, Guilloux-Benatier M, Alexandre H, Feuillat M (2001) Influence de l'autolyse des levures apres fermentation sur le développement de Brettanomyces-Dekkera dans le vin. J Int Sci Vigne Vin 35:157–164
109. Gerós H, Azevedo MM, Cássio F (2000) Biochemical studies on the production of acetic acid by the yeast Dekkera anomala. Food Technol Biotech 38:59–62
110. Baumes R, Cordonnier R, Nitz S, Drawert F (1986) Identification and determination of volatile constituents in wines from different vine cultivars. J Sci Food Agr 37:927–943
111. Schreier P, Drawert P, Abraham KO (1980) Identification and determination of volatile constituents in Burgundy Pinot noir. LWT-Food Sci Technol 13:318–321
112. Etievant PX (1981) Volatile phenol determination in wine. J Agr Food Chem 29:65–67
113. Chatonnet P, Dubourdieu D, Boidron JN, Pons M (1992) The origin of ethylphenols in wines. J Sci Food Agr 60:165–178
114. Edlin DAN, Narbad A, Gasson MJ, Dickinson JR, Lloyd D (1998) Purification and characterization of hydroxycinnamate decarboxylase from Brettanomyces anomalus. Enzyme Microb Tech 22:232–239
115. Chatonnet P, Dubourdieu D, JN Boidron, Lavigne V (1993) Synthesis of volatile phenols by Saccharomyces cerevisiae in wines. J Sci Food Agr 62:191–202
116. Barthelmebs L, Divies C, Cavin J-F (2000) Knockout of the p-coumarate decarboxylase gene from Lactobacillus plantarum reveals the existence of two other inducible enzymatic activities involved in phenolic acid metabolism. Appl Environ Microb 66:3368–3375
117. Godoy L, Garrido D, Martínez C, Saavedra J, Combina M, Ganga MA (2009) Study of the coumarate decarboxylase and

vinylphenol reductase activities of Dekkera bruxellensis (anamorph Brettanomyces bruxellensis) isolates. Lett Appl Microbiol 48:452–457
118. Tchobanov I, Gal L, Guilloux-Benatier M, Remize F, Nardi T, Guzzo J, Serpaggi V, Alexandre H (2008) Partial vinylphenol reductase purification and characterization from Brettanomyces bruxellensis. FEMS Microbiol Lett 284:213–217
119. Tchobanov I (2007) Sélection de souches de levures Bulgares. Purification partielle et caractérisation de la vinylphénol réductase chez Brettanomyces. Thèse de doctorat de l'universite de Bourgogne, France
120. Froudière I, Larue F (1988) Conditions de survie de Brettanomyces (Dekkera) dans le moût de raisin et le vin. Connaiss Vigne Vin 2:296–303
121. Boulton RV, Singleton L, Bisson R, Kunke E (1996) The maturation and ageing of wine. Principles and practices of winemaking. Chapman and Hall, New York, pp 382–426
122. Smith CR (1996) Studies of sulfur dioxide toxicity for two wine yeasts. University of California, Davis
123. Izquierdo-Cañas PM, García-Romero E, Huertas-Nebreda B, Gómez-Alonso S (2012) Colloidal silver complex as an alternative to sulphur dioxide in winemaking. Food Control 23:73–81
124. McMurrough I, Madigan D, Donnelly D, Hurley J, Doyle AM, Hennigan G, McNulty N (1996) Control of ferulic acid and 4-vinyl guaiacol in brewing. J Inst Brewing 102:327–332
125. Sims CA, Eastridge JS, Bates RP (1995) Changes in phenols, color, and sensory characteristics of Muscadine wines by pre- and post-fermentation additions of PVPP, casein, and gelatin. Am J Enol Vitic 46:155–158
126. Donovan J, McCauley J, Tobella N, Waterhouse AL (1999) Effects of small-scale fining on the phenolic composition and antioxidant activity of Merlot wine. In: WaterHouse AL (ed) Wine Chemistry. ACS Symposium Series, Washington DC, pp 142–155
127. Lisanti MT, Piombino P, Gambuti A, Genovese A, Moio L (2008) Oenological treatments for the removal of geosmin, responsible for earthy off-flavour, in wine. XXXIst World Congress of Vine and Wine. Verona, Italy, pp 15–20
128. Guzzon R, Nardin T, Micheletti O, Nicolini G, Larcher R (2013) Antimicrobial activity of ozone. Effectiveness against the main wine spoilage microorganisms and evaluation of impact on simple phenols in wine. Aust J Grape Wine Res doi. doi: 10.1111/ajgw.12018
129. Cantacuzene NO, Dormedy ES, Smilanick JL, Fugelsang KC, Wample RL, et al. (2003) Treating Brettanomyces in oak cubes with gaseous and aqueous ozone. ASEV 54th annual meeting Reno, Nevada
130. Coggan M (2003) Ozone in wineries. http://www.ciprocess.co.uk/pdfs/Ozone_In_Wineries.pdf
131. Renouf V, Strehaiano P, Lonvaud-Funel A (2008) Effectiveness of dimethyldicarbonate to prevent Brettanomyces bruxellensis growth in wine. Food Control 19:208–216
132. Costa A, Barata A, Malfeito-Ferreira M, Loureiro V (2008) Evaluation of the inhibitory effect of dimethyl dicarbonate (DMDC) against wine microorganisms. Food Microbiol 25:422–427
133. Gómez-Rivas L, Escudero-Abarca B, Aguilar-Uscanga MG, Hayward-Jones P, Mendoza P, Ramírez M (2004) Selective antimicrobial action of chitosan against spoilage yeasts in mixed culture fermentations. J Ind Microbiol Biotechnol 31:16–22
134. Bornet A, Gautier S, Teissedre PL (2008) Elimination des goûts terreux (la géosmine) et des Brettanomyces par l'utilisation d'un biopolymère fongique: le chitosane. The XXXI World Congress of Vine and Wine, Verona
135. Larcher R, Puecher C, Rohregger S, Malacarne M, Nicolini G (2012) 4-Ethylphenol and 4-ethylguaiacol depletion in wine using esterified cellulose. Food Chem 132:2126–2130
136. Santos A, Navascués E, Bravo E, Marquina D (2011) Ustilago maydis killer toxin as a new tool for the biocontrol of the wine spoilage yeast Brettanomyces bruxellensis. Int J Food Microbiol 145:147–154
137. Enrique M, Marcos JF, Yuste M, Martínez M, Vallés S, Manzanares P (2008) Inhibition of the wine spoilage yeast Dekkera bruxellensis by bovine lactoferrin-derived peptides. Int J Food Microbiol 127:229–234
138. Benito S, Palomero F, Morata A, Uthurry C, Suárez-Lepe JA (2009) Minimization of ethylphenol precursors in red wines via the formation of pyranoanthocyanins by selected yeasts. Int J Food Microbiol 132:145–152
139. Morata A, Vejarano R, Ridolfi G, Benito S, Palomero F, Uthurry C, Tesfaye W, González C, Suárez-Lepe JA (2013) Reduction of 4-ethylphenol production in red wines using HCDC + yeasts and cinnamyl esterases. Enzyme Microb Technol 52:99–104
140. Santos A, San Mauro M, Bravo E, Marquina D (2009) PMKT2, a new killer toxin from Pichia membranifaciens, and its promising biotechnological properties for control of the spoilage yeast Brettanomyces bruxellensis. Microbiology 155:624–634
141. Chassagne D, Guilloux-Benatier M, Alexandre H, Voilley A (2005) Sorption of wine volatile phenols by yeast lees. Food Chem 91:39–44
142. Haslam E, Lilley TH, Warminski E, Liao H, Cai Y, Martin R, Gaffney-Simon H, Goulding-Paul N, Luck G (1992) Polyphenol Complexation. Phenolic Compounds in Food and Their Effects on Health I: American Chemical Society, Chapter 2:8–50
143. Kawamoto H, Nakatsubo F (1997) Effects of environmental factors on two-stage tannin-protein co-precipitation. Phytochemistry 46:479–483
144. Salmon JM, Fornairon-Bonnefond C, Mazauric JP (2002) Interactions between wine lees and polyphenols: influence on oxygen consumption capacity during simulation of wine aging. J Food Sci 67:1604–1609
145. Caridi A, Sidari R, Solieri L, Cufari A, Giudici P (2007) Wine colour adsorption phenotype: an inheritable quantitative trait loci of yeasts. J Appl Microbiol 103:735–742
146. Couto JA, Barbosa A, Hogg T (2005) A simple cultural method for the presumptive detection of the yeasts Brettanomyces/Dekkera in wines. Lett Appl Microbiol 41:505–510
147. Calderón F, Morata A, Uthurry C, Suárez JA (2004) Aplicaciones de la ultrafiltración en la industria enológica. Últimos avances tecnológicos. Tecnología del Vino 16:49–54
148. Umiker NL, Descenzo RA, Lee J, Edwards CG (2012) Removal of Brettanomyces bruxellensis from red wine using membrane filtration. J Food Process Preserv. doi:10.1111/j.1745-4549.2012.00702.x
149. Vuchot P, Favre E, Noilet P, Barnavon L (2007) Le traitement des vins phénolés: aspects curatifs. Proceedings XXXth OIV World Congress, Budapest
150. Palomero F, Ntanos K, Morata A, Benito S, Suárez-Lepe J (2011) Reduction of wine 4-ethylphenol concentration using lyophilised yeast as a bioadsorbent: influence on anthocyanin content and chromatic variables. Eur Food Res Technol 232:971–977
151. Puértolas E, López N, Condón S, Raso J, Álvarez I (2009) Pulsed electric fields inactivation of wine spoilage yeast and bacteria. Int J Food Microbiol 130:49–55
152. Lustrato G, Vigentini I, De Leonardis A, Alfano G, Tirelli A, Foschino R, Ranalli G (2010) Inactivation of wine spoilage yeasts Dekkera bruxellensis using low electric current treatment (LEC). J ApplMicrobiol 109:594–604

153. Schmid F, Grbin P, Yap A, Jiranek V (2011) Relative efficacy of high-pressure hot water and high-power ultrasonics for wine oak barrel sanitization. Am J Enol Vitic 62:519–526
154. Morata A, Benito S, González MC, Palomero F, Tesfaye W, Suárez-Lepe JA (2012) Cold pasteurisation of red wines with high hydrostatic pressure to control Dekkera/Brettanomyces: effect on both aromatic and chromatic quality of wine. Eur Food Res Technol 235:147–154
155. González-Arenzana L, Santamaría P, López R, Garijo P, Gutiérrez AR, Garde-Cerdán T, López-Alfaro I (2013) Microwave technology as a new tool to improve microbiological control of oak barrels: a preliminary study. Food Control 30:536–539
156. Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D (2006) Effect of the type of barrel on the development of red wine. In John Wiley & Sons (eds) Handbook of enology. The chemistry of wine stabilization and treatments, Vol. 2. Chichester, England