Chapter

Wine Stabilisation: An Overview of Defects and Treatments

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Abstract

Wine is widely consumed due to its distinctive sensory characteristics. However, during wine production and storage, several defects can appear. These can be the result of unwanted microbiological activity or due to the unbalanced levels of some compounds resulting from an unbalanced grape chemical composition or inadequate winemaking practices and storage conditions. The main purpose of wine stabilisation is the removal of wine defects, either visual, olfactive, gustative, or tactile, the increase in wine safety and stability by fining and filtration operations, avoiding the occurrence of some usual wine precipitations after bottling. Although the best strategy is to prevent the appearance of wine defects, when present, several fining agents or additives, and technologies are available today with different performances and impact on wine quality. By physicochemical and sensory analysis, the defect is detected, and if the objective is removing them, some laboratory trials are performed to achieve a better treatment approach. This review overviews the principal wine defects and treatments available today and in the near future. Generally, the future trend is the use of more sustainable and environmentally friendly fining agents and technologies, looking for treatments with better performance and specificity.

Keywords: wine defects, stabilisation, wine treatments, fining agents

1. Introduction

All around the world in wine production many sensory defects, physicochemical instabilities, and a few toxic compounds can appear, which usually results in a decrease of the wine quality and/or safety, being responsible for economic losses for the wine industry. Nowadays, the origin of most wine defects and processing conditions that favour their formation are well identified. At the same time, many viticultural and technological solutions are available to completely or partially avoid their formation, and various additives and fining agents are allowed to be used to avoid their formation or remove the compounds, or their precursors, responsible for the instability [1]. Frequently, the question is not how to remove the defect or their precursors, but how to remove it without changing the wine sensory profile.

The most troublesome defects for wine producers are those occurring after wine bottling, as the intervention at this stage is rather limited and therefore preventive actions are the most efficient and sometimes the only strategy. After wine bottling the main external factor that can influence wine stability and the kinetics of the chemical reactions and interactions of the wine constituents is the temperature that can significantly affect for example the solubility of tartrate salts, induce colour

changes in red and white wines, and the formation of protein haze. Also, the redox potential, mainly determined by the levels of dissolved oxygen in the wine can significantly influence the wine shelf life. Before bottling, depending on the wine defect to be treated two main stabilisation strategies are currently used: subtractive and non-subtractive. For the first approach fining agents are used that can interact with the compound(s) responsible for the wine defect or their precursors removing them from the wine. In the second type of strategy, an additive is added to the wine that interacts with the compound(s) responsible for the wine defect affecting their ability to form crystals as in the case of carboxymethylcellulose in the tartaric instability [2] or decrease the vapour pressure of the compound(s) responsible for an undesirable odour for example like chitosan in the case of the ethylphenols responsible for the '*Brett character*' [3].

In the next sections (2, 3, 4, and 5) the main wine defects and their stabilisation treatments currently allowed and used will be overviewed as well as the stabilisation treatments currently under research. For a matter of simplicity and systematisation, the wines defect that can occur in wine production are divided into four main groups according to their impact on the wine sensory quality and safety: (1) visual sensory defects; (2) off-odours and taints; (3) taste and tactile defects (4) safety-related defects. The wine visual defects are concerned with changes in wine limpidity and undesirable colour changes that can occur during wine production and especially after wine bottling. These defects can be due to the formation of precipitates related to solubility issues, the formation of precipitates related to the colloidal instability of some wines components, the reaction between wine components, or due to the oxidation of wine phenolic compounds.

Wine aroma significantly determines consumer acceptability [4], being extremely complex as it is the result of the cumulative effect of a diverse group of volatile compounds present at levels ranging from fractions of ng/L up to mg/L. These compounds can interact with the olfactory epithelium to generate a sensory perception [5, 6]. The levels of these volatiles are dependent on both viticultural [7] and oenological practices [8, 9]. Although several hundreds of these volatile compounds can be present in wines [10], only a few are present at levels above the perception threshold and thus being responsible for characteristic odours [11]. During wine production, several off-odours and taints can cause severe quality problems. Off-odours are considered to be the occurrence of any atypical odours resulting from compounds formed by the deterioration of the wine, including chemical reactions and microbial spoilage; whereas, taints result from external contamination of the wine as a result of exposure of grapes to contaminating environments or migration of compounds form packaging materials contaminated by either synthetic chemicals or chemicals produced or transformed by microbial action [12]. Sometimes, compounds that impart a positive aroma character in one instance may cause an off-odour when present in the wrong context or at high levels. Besides, faults in one wine may not be undesirable in another: for example, the complex oxidised bouquets of Sherries, the fusel odours of Port wine, and the baked character of Madeira wine. Some faults, such as a barnyard odour, generated by ethylphenols, may be considered pleasingly 'rustic,' or part of the terroir character of certain wines. The evident presence of ethyl acetate in the aroma of wine is also usually considered a fault. However, in expensive Sauternes, it appears to be acceptable (or ignored). Nevertheless, there is general agreement among most wine professionals as to what constitutes an aromatic fault in table wines. These wine defects, their origin, and their sensory impact are discussed in Section 2. Balancing the oral sensations of wine is one of the most demanding tasks for a winemaker since a distinguishing feature of superior wines is the harmony achieved among these seemingly simple sensations. Indeed, imbalances created by excessive acidity,

astringency, or bitterness, among others, are often the first deficiencies noted by a panellist [13]. Since it is very important to understand the factors that affect the sensory properties responsible for wine taste and tactile imbalance, to allow the wine industry to be able to control these sensory characteristics of the final product from the first production stages. The main wine taste and tactile defects are astringency, bitterness, and acidity imbalance. Wine astringency is caused by excess proanthocyanidins in young red wine, it is considered a tactile sensation [14], responsible for the 'drying', 'roughing' and 'puckering'. The ageing process reduces astringency due to oxidation and will be less evident in aged wines. Bitterness is a taste perception related to phenolic compounds with low molecular weights such as flavonol aglycones, especially myricetin or quercetin [15] as well as to monomeric or small phenolic flavanols [16]. Bitterness, astringency, and acidity could change depending on the oenological practice. In Section 4 the sensory impact of excessive astringency, bitterness and acidity will be highlighted, as well as the stabilisation/correction strategies.

In Section 5, the formation of the main potential toxic compounds that affect wine safety is overviewed. These compounds are present in wines due to the activity of bacteria and moulds in the wine production and due to the bad hygienic sanity of the grapes used for winemaking, respectively.

2. Origin of visual sensory defects and strategies for wine stabilisation

The two most important visual sensory defects that affect wine quality occur during the storage of bottled wine. These are the tartrate instabilities and protein instability. Other instabilities that can occur include red wine colour colloidal instability, pinking of certain white wines produced from white grape varieties, browning of white and rose wines, and oxidation of all wines as well as metallic instabilities such as iron and copper instability.

2.1 Tartrate instabilities

The crystallisation of potassium hydrogen tartrate or calcium tartrate salts may occur in wines where their concentration product exceeds their solubility product [17, 18]. The formation of these crystals results in the formation of deposits at the bottom vat and sometimes in the bottled wine. Prevention of tartaric precipitation in bottled wine is essential as consumers associate its occurrence with poor production conditions or an exogen wine material. Methods to prevent tartaric crystals precipitation include metatartaric acid, cold stabilisation, and electrodialysis [19]. The addition of mannoproteins obtained from the hydrolysis of the yeast cell wall was authorised by the European Community since 2005 [19]. Mannoproteins inhibit the crystallisation of tartrate salts by lowering the crystallisation temperature [20] preventing the occurrence of precipitates in wine [21]. Arabic gum can also have some effectiveness to prevent tartaric instability, as they are protective colloids [22], and more recently sodium carboxymethylcellulose for white wine (since 2009) and potassium polyaspartate (since 2015) were authorised [19].

2.2 Protein instability

Precipitation of soluble proteins in bottled wines can also occur and this results in the formation of an amorphous haze or deposit. This defect occurs frequently in white wines or wines with low polyphenol content. It is rare in wines with relatively high levels of flavonoid phenols, particularly tannins, which complex and

precipitates proteins during wine production. The most important proteins that have been related to wine protein instability are pathogenesis-related proteins of *Vitis vinifera* that include the chitinases and thaumatin-like proteins as described by Tian et al. [23]. The formation of wine protein haze is a multifactorial process with several factors known to influence the process, such as storage or wine ageing temperature, pH, ionic strength, wine protein composition, organic acids, ethanol, phenolic compounds, metals, and sulphate content; however, other important factors remain unidentified, such as the non-proteinaceous component(s) usually named X factor [24]. These proteins can be slowly denatured and aggregate throughout wine storage, forming a light-dispersing haze; therefore, this phenomenon needs to be prevented by removing them from the wine, usually by fining, before wine bottling [25]. Bentonite fining is the most used process to avoid protein instability in white wine, with the dose used being preferentially determined previously by stability tests [25]. However, bentonite fining can have a detrimental effect on wine quality, for example, by the removal of colour and aroma compounds [26]. Therefore, alternative techniques to bentonite fining have been studied, such as ultrafiltration, the addition of proteolytic enzymes, flash pasteurisation, other adsorbents, zirconium oxide, natural zeolites, chitin and chitosan, carrageenan and the use of some mannoproteins [25].

2.3 Colour colloidal instability

Colour instability of some red wines can cause product depreciation. In the last years, excessive precipitation of colouring matter has been observed at the bottom of the storage tanks and even in bottled wines [27]. This precipitation occurs along with the natural evolution of red wine during the storage period and results in a considerable modification of its colour and limpidity that is undesirable in terms of visual perception and loss in taste and flavour [22]. This precipitate may happen after a few months of ageing. It may be sometimes gelatinous and strongly red coloured. If the red wine is aged and bottled, the precipitate may occur later in the form of a thin leaf, lining the inner side of the bottles [28]. A method commonly used to reduce wine turbidity and stabilise the colouring matter is fining by the addition of proteins (albumin, casein, or gelatine) that promotes flocculation or precipitation before bottling [29]. However, in some cases, the precipitate formation can still be observed in the later stages of the ageing process. The addition of arabic gum could prevents wine colour instability [22].

2.4 Metallic instabilities

Two metal-dependent instabilities can also result in the formation of wine turbidity or deposits. Excess levels of iron (5–20 mg/L) in wine (white or red) can lead to the formation of precipitates with phosphates and tannins resulting in instabilities (iron (III) phosphate [white casse] or tannate [blue casse]). Application of arabic gum or casein/potassium caseinate or citric acid (< 1 g/L) is used to prevent this instability [30]. Copper instability occurs only in white wines, initially as a white haze, and later as a reddish-brown amorphous precipitate, develop upon storage of bottled wine with excess copper (> 0.5 mg/L), under strong reducing conditions and in the presence of SO_2. This metal instability is caused by a reaction of metal traces, mainly copper from machinery, pesticide residues, or treatment with copper sulphate for the treatment of reductive off-flavours [31]. The OIV recommends a maximum copper content of 1.0 mg/L in wines [32]. The application of arabic gum is used to prevent this instability [30]. The protective colloids prevent metal precipitation but do not eliminate the copper. Exchange resins such

as polyvinylimidazole-polyvinylpyrrolidone copolymers with selective binding of metals such as copper or iron have been developed [33]. The occurrence of iron and copper-related precipitations are much less usual due to the reduction of the level of these metals in the wines all-around the world by the use of stainless steel vats and wood barrels in the wine production, vat taps, and plastic polymers in the winery hoses, press machines and filling machines.

2.5 Enzymatic and non-enzymatic oxidation

One of the most frequent oenological problems in winemaking is premature wine oxidation, especially the oxidative spoilage of young white wines causing wine browning [34]. During winemaking and bottle-ageing wine, components react with oxygen [35]. Moderate oxidation of red wines phenolic compounds can contribute positively to the red wine colour stabilisation and decrease wine astringency, nevertheless, excessive oxidation can have negative effects on wine quality [36]. Wine oxidation generally results in wine colour changes, an important sensory attribute that is the first to be appreciated by consumers. Today the market wants white wines with a citrine colour, almost colourless, except for those white wines fermented in oak barrels or wines with some ageing time. In rosé wines, many colours can be found on the market, since the 'Provence style', with a slight salmon colour, until rosé wines with the colour of open coloured red wines like 'Palhete', wines produced with white and red grapes and with some maceration. In the red wines, many styles and colours can be found, from the faint colour of Pinot Noir wines to the wines produced with Alicante Bouschet or Vinhão grape varieties that yield wines with intense red colours. The fast colour change in a white or rosé bottled wine is normally the result of an oxidative problem.

The deleterious browning reaction in must and wine occurs due to the oxidation of phenolic compounds and can start as soon the grapes are crushed due to the polyphenol oxidase activity. Polyphenol oxidase with tyrosinase and catecholase activity are natural enzymes present in grape berry. They can catalyse the oxidation of monophenols to *o*-diphenols and further oxidation to orthoquinone. In wine hydroxycinnamates and flavanols, such as caffeoyltartaric acids and catechin, respectively [37] are oxidised to the corresponding quinones. Further reaction of the quinones can result in the formation of a brown colour, especially that of catechin than can yield by dimerisation the yellow dehydrodicatechin B [38] Another problem can arise when grapes are affected with *Botrytis cinerea* [39] and the resulting must become contaminated with laccase enzyme. Laccase catalyses the one-electron oxidation of a broad range of compounds including substituted phenolics to the corresponding radicals [40]. Wine phenolic acids, catechins, anthocyanins, tannins, and stilbenes are converted into the corresponding quinones, which often react further to dark coloured polymers [41]. The latter are generally insoluble in water and precipitate out from must and wine. Grape polyphenol oxidase is sensitive to low concentrations of SO_2 being inactivated, but laccase is more resistant to SO_2, and it may be present in the final wine [22], while polyphenol oxidase rarely survives the fermentation process [42]. After fermentation, with the enzyme removed or inactivated, oxidation reactions in white wine are based on non-enzymatic pathways, where Fe (II) is oxidised to Fe (III), producing hydrogen peroxide, and the following reaction where Fe (III) coordinates with catechols and oxidises them to semiquinones [43]. Then the semiquinones disproportionate to form reactive electrophilic quinones and these reactive compounds have a key impact on wine chemistry, by degrading several colour and flavour substances [35]. Reactions of oxidation products with flavonoids are well known, and some of the products are pigmented. When tartaric acid is oxidised to glyoxal, the resulting bridged product

continues to react, creating a xanthylium product that absorbs in the visible region, and may contribute to the yellow hue of oxidised wines [44].

To avoid the fast colour evolution, the winemakers use SO_2 that due to their antioxidant and antioxidasic properties protect wine colour [45]. Unlike grape oxidases, which are inhibited by sulphites even at low levels, fungal laccases tend to be more resistant. The most effective treatment to eliminate the laccase activity in the must is heat treatment (2 min, 75°C). Ascorbic acid reduces and recycles quinones back to their original catechol forms, being generally used in pre-bottling. The presence of other nucleophiles, such as glutathione, 3-sulfanylhexanol and H_2S, leads to the formation of additional products on different positions of the benzene ring [45], and such reactions should also prevent browning since the quinone is being quenched. There are also many technological solutions that when used can results in a more stable wine colour as, in white wines, a fast liquid/solid separation in the press machines, reducing phenolic acids by wine fining with PVPP, potassium caseinate/casein, isinglass, gelatine, patatin and pea protein. Winemakers need to be especially cautious when handling a cold wine, such as during cold stabilisation. Oxygen is more soluble at lower wine temperatures. However, the oxidation reaction speeds up when the temperature rises. As the cold wine warms up the greater amount of dissolved oxygen will contribute to serious wine oxidation. To minimise the adverse effects of oxidation during wine racking the winemakers employ several techniques such as, using SO_2, using gentle pumps that minimise aeration, and checking hoses and fittings for leaks, and flushing hoses and containers with inert gas before wine racking. In modern winemaking, the inert gases are often used to minimise oxygen pickup in the head space of partially filled containers and during wine racking. The common inert gases used include; nitrogen, CO_2, argon, and a mixture of these gases in various proportions. For economic reasons, the use of nitrogen and CO_2 seems to be more common. To provide an inert gas cover over the wine surface in a partially filled container, CO_2 or argon should be used. These gases are denser than the air and form an inert layer devoid of oxygen. The danger of oxygen exposure is greater during the wine racking. To minimise oxygen/air contact the system is purged with the inert gas. In the process of purging the inert gas is passed through the system such as hoses, transfer lines, equipment, and the receiving tank to displace air. The wine is then racked under an inert atmosphere.

2.6 Pinking

The development of a salmon-red blush colour in white wines produced exclusively from white grape varieties is known as pinking, and the phenomenon is observed occasionally. It is perceived as an undesirable phenomenon by both wine consumers and the industry. Although with seasonal and regional variations, pinking has been observed worldwide, with predominance in white wines produced from *V. vinifera* L. grape varieties such as Chardonnay, Chenin Blanc, Crouchen, Muscat Gordo Blanco, Palomino, Riesling, Sauvignon Blanc, Sémillon, Sultana, and Thompson Seedless [46]. Pinking is mainly observed when white wines are produced under reducing conditions [47]. The pinking phenomenon is frequently observed after the bottling and storage of white wines or after alcoholic fermentation (AF) [48]. In wines made from Síria white grape variety, it was shown that the compounds responsible for the appearance of the salmon colour after bottling were due to the presence of small amounts of anthocyanins in the wine that could also be detected both in the pulp and in the skin of the white grapes [46].

Although it cannot be excluded that other compounds can be responsible for the appearance of a salmon colour in white wines from other grape varieties, the presence of the small number of anthocyanins in Chardonnay, Sauvignon Blanc,

and Riesling has been shown [49]. To avoid the pinking problem there are various preventive or curative oenological treatments, including adding PVPP or PVPP associated with bentonite or increasing the redox potential using ascorbic acid in the pre-bottling stage [46].

3. Off-odours and taints and strategies for their mitigation and wine stabilisation

Uncontrolled or undesirable microbiological activity developed in the wine can be responsible for several wine spoilage problems. These defects are diverse in origin and chemical compounds involved impact as well on the wine sensory quality.

3.1 Wine off-odours

One of the main problems that can occur is the development of high levels of volatile acidity, mainly acetic acid (I, **Figure 1**). 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 [50]. High volatile acidity is associated with bad SO_2 management or extreme wine exposure to oxygen that stimulate the growth of aerobic acetic acid bacteria (AAB), that increases acetic acid. This results in an olfactory sensory defect known as vinegar off-odour. Vinegary wines are typically sharply acidic with an irritating odour. Ideally, the content of acetic acid should not exceed 0.7 g/L in wine. Several methodologies, aiming to decrease excessive volatile acidity of acidic wines have been proposed [50], such as microbial stabilisation of the acidic wine followed by blending with other wines, reverse osmosis, nanofiltration, and biological removal of acetic acid through refermentation [22].

Acetaldehyde (ethanal) (II, **Figure 1**) in wine can impart some undesirable flavours, when above a certain level. The average values of acetaldehyde in white wine are about 80 mg/L, in red wine 30 mg/L and for Sherries wine 300 mg/L [51]. Acetaldehyde is an intermediate product of yeast fermentation; however, it is more commonly associated with ethanol oxidation, catalysed by the enzyme ethanol

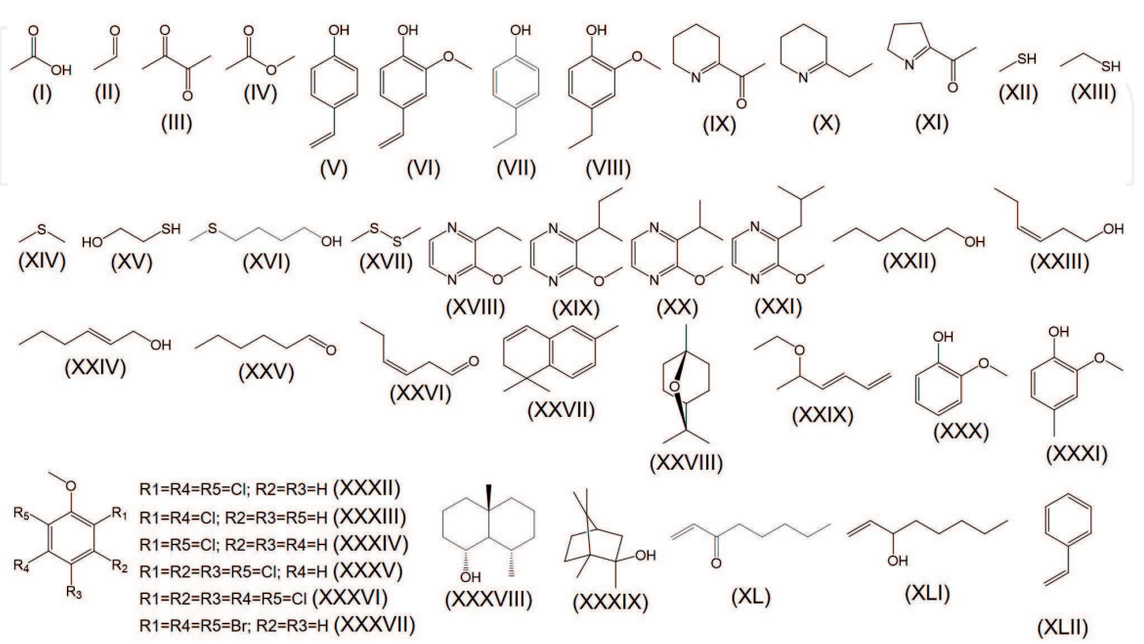

Figure 1.
Structure of the main wine off-odours and taints.

dehydrogenase. Moreover, acetaldehyde can be formed by non-enzymatic oxidation throughout the storage and ageing of wine [52]. During wine oxidation, iron (II) reduces oxygen to the hydroperoxyl radical, which converts wine *ortho*-diphenols phenols into quinones and H_2O_2. Ferrous ion associated with H_2O_2 generates hydroxyl radical that can react with ethanol to yield acetaldehyde [53]. The sensory threshold for acetaldehyde in red wines is typically in the range of 40–100 mg/L [54]. If present at low levels gives a pleasant fruity aroma, but at high levels, it possesses a pungent irritating odour [55]. Indeed, excess acetaldehyde produces a 'green,' 'grassy,' 'nutty,' 'sherry-like,' 'bruised apple,' or even 'vegetative' off-flavour [30, 56]. The level of acetaldehyde in wine can be reduced by appropriate yeast strain selection, as well as the prevention of oxidation during the winemaking process [57]. The reduction of acetaldehyde can also be done by wine lactic acid bacteria (LAB) of the genera *Lactobacillus* and *Oenococcus* which can degrade free and SO_2-bound acetaldehyde [58]. Acetaldehyde also strongly binds to SO_2, reducing the free acetaldehyde content, and thus the perception of its aroma in wines [2].

Diacetyl (2,3-butanedione, III in **Figure 1**), is usually found in low levels, as a result of yeast metabolism (<1 mg/L), but it is principally formed during malolactic fermentation (MLF), by the metabolism of citric acid, which is usually naturally present in wines at levels between 0.1–0.7 g/L [59]. If present in an excessive content sufficient to affect wine's flavour, is usually considered as a fault, generating a buttery, nutty or toasty, lactic off-odour. The detection threshold for diacetyl in a 10% aqueous ethanol is 0.1 mg/L [11]. However, the diacetyl detection threshold is dependent on the wine matrix. It has been reported as 0.2 mg/L in white wine (Chardonnay) and from 0.9 mg/L (Pinot Noir) to 2.8 mg/L (Cabernet Sauvignon) in red wines [60]. Also, diacetyl quickly bounds SO_2, and the free and bound forms of diacetyl are in chemical equilibrium, depending on the pH, the level of SO_2, and the presence of other SO_2 binding components, such as acetaldehyde, α-ketoglutaric acid, and pyruvic acid are important [61]. It is assumed that only the unbound form of diacetyl is sensorially active. According to Nielsen and Richelieu [61] the addition of 80 mg SO_2, which is within the range used in the wine industry, reduced the free diacetyl content (20 mg/L) by 75%.

All wines contain a few tens of mg/L of ethyl acetate (30–60 mg/L, IV in **Figure 1**) produced by yeast, higher levels indicate AAB activity, formed by esterification between acetic acid and ethanol. This compound at low levels in wine (<50 mg/L) may not be unpleasant, contributing to 'fruity' aroma properties and add complexity to the wine, but at levels >150 mg/L ethyl acetate can confer an unpleasant 'fingernail polish' aroma [62]. Ethyl acetate has a perception threshold in the wine of around 160–180 mg/L, which is much lower than that of acetic acid (750 mg/L) [22]. The deleterious effect of ethyl acetate can be in part reduced by ageing [63] but, after 6 months of bottle ageing, the ethyl acetate levels (140–180 mg/L) affect the wine flavour, giving wines a hot flavour which reinforces the impression of bitterness on the aftertaste [22]. It is usually more perceived in white wine than red wines. Factors that can influence ethyl acetate formation include the yeast strain used during the AF as well as the temperature of fermentation and SO_2 levels. Ethyl acetate is also produced by AAB and is related to dissolved oxygen levels in the wine [64].

Vinylphenols and ethylphenols are collectively known as volatile phenols (VPs). Vinylphenols (4-vinylphenol and 4-vinylguaiacol, V, and VI in **Figure 1**, respectively) are produced by the yeast *S. cerevisiae*, LAB such as *L. plantarum*, and *Dekkera/Brettanomyces* yeasts [65]. Their impact on wine quality is almost exclusively observed in white wines, as these wines can contain significant quantities of vinylphenols which, beyond a certain content (limit threshold = 725 μg/L of 4-vinylguaiacol+4-vinylphenol (1:1)), can be responsible for a depreciating

'phenolic' or 'pharmaceutic' characteristic [66]. *S. cerevisiae* possesses a cinnamate carboxylase enzyme which can transform by non-oxidative decarboxylation, the phenolic acids *p*-coumaric and ferulic acids, into corresponding vinylphenols. This activity is only expressed during AF and with a variable intensity depending on the yeast strain. Although *Dekkera/Brettanomyces* yeasts can also produce vinylphenols they are more likely to reduce the available vinylphenols to ethyl derivatives. It has been shown that *Dekkera/Brettanomyces* is the only known microorganism that under winemaking conditions can produce significant amounts of VPs [67]. The ethylphenols are formed by these yeasts through decarboxylation of the corresponding hydroxycinnamic acids to vinylphenols, and subsequent reduction to ethylphenols, yielding 4-ethylphenol (VII in **Figure 1**) from *p*-coumaric acid and 4-ethylguaiacol (VIII in **Figure 1**) from ferulic acid [67, 68]. Some attributes, such as animal, stable, horse sweat was designated by the widespread term '*Brett-character*' in oenology [69]. The perception threshold of EPs (4-ethylphenols, designated as 4-ethylphenol and 4-ethylguaiacol) is influenced by the wine matrix. The values reported by Chatonnet et al. [68] 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 [70] while the combination of 4-ethylphenol with 4-ethylguaiacol shows a threshold of 400 μg/L [68]. Nowadays, perhaps it is the most problematic sensory defect in red wine production around the world, with million litres being be contaminated each year [71]. In the last years, research has been performed to remove these negative VPs from contaminated red wines [67] and efficient treatments include activated carbons (ACs) and fungal chitosan to avoid the growth of contaminated yeast or to reduce the head space volatility of these negative VPs [3, 72]. New materials have been evaluated for their removal aiming to decrease the negative impact of the former treatments on wine quality. Of the new material that includes molecularly imprinted polymers [73], chitosan [3] and degassed and ethanol impregnated cork powder [74], that can remove about 70% of ethylphenols allowing a significant recovery of the wine's fruit and floral character [74]. This material is cheap and easily prepared from cork powder wastes, being natural with good biodegradability, and low environmental impact.

The formation of mousy off-flavours can occur during (MLF) either by the action of LAB (particularly heterofermentative strains) or *Dekkera/Brettanomyces* yeast. This off-flavour can be associate to three compounds, namely the N – heterocyclic volatile bases 2-acetyltetrahydropyridine (sensory threshold in water =1.6 μg/L, IX, **Figure 1**), 2-ethyltetrahydropyridine (odour threshold in wine = 150 μg/L, X, **Figure 1**, [75] and 2-acetylpyrroline (detection threshold in water = 0.1 μg/L, XI, **Figure 1**, [76], being the first one produced at the highest levels. *Dekkera/Brettanomyces* are capable of producing at least two of these compounds, whereas LAB are capable to produce all the three [77]. Although the biosynthetic pathway for the mousy off-flavour compounds formation in wine is unknown, the conditions necessary for its production have been established. L-lysine and L-ornithine are the precursors of the heterocycle ring of the three mousy compounds, and ethanol and acetaldehyde are responsible for the acetyl side chain. The presence or absence of certain metal ions and oxygen has a substantial effect on off-flavour production [77]. However, there is still not know efficient treatment to remove the mousy off-flavour from wines [78]. At present research studies are being performed on the use of molecular imprinting technology for developing materials with the capacity to selectively remove the mousy off-odour [79]. Therefore, at present, it is necessary to prevent the biosynthesis of the mousy off-odour-forming compounds, by the elimination or strict control of the yeast and bacteria responsible for their formation. This can be achieved by implementing effective microbial control strategies in the winery [77].

Aroma properties evocative of rotten eggs, cabbage, garlic, putrefaction are termed 'reduction'. These aroma attributes are generally considered to contribute negatively to overall wine sensory quality and are considered to be related to different low molecular weight volatile sulphur compounds, such as H_2S, (odour threshold in red wine 1.1 μg/L), methyl mercaptan (methanethiol, odour threshold in red wine 1.8 μg/L, XII, **Figure 1**), ethyl mercaptan (ethanethiol, odour threshold in red wine 1.1 μg/L, XIII, **Figure 1**), and dimethyl sulphide (odour threshold in red wine 25 μg/L, XIV, **Figure 1**) [80]. Yeast fermentation is frequently associated with the occurrence of reductive off-odours, mainly linked to the formation of H_2S and mercaptan by the yeast as mentioned by Pereira et al. [81]. As nitrogen availability is considered one of the main factors for H_2S production by yeast, a strategy that could be adopted is the addition of yeast assimilable nitrogen to supplement fermentation [80]. The production of H_2S during the AF is normal and the quantity produced is dependent on multifactorial factors such, yeast DNA, grape juice turbidity, level of assimilable nitrogen in the grape juice, levels of methionine and cysteine, fermentation temperature, high levels of SO_2, and sulphates. This type of aroma sometimes masks completely the positive varietal and fermentative aroma, however, H_2S is very volatile and usually, simple wine aeration is enough to remove them or can be precipitated with copper sulphate or copper citrate. The excessive aeration of the wine in the presence of H_2S could lead, by oxidation, to the production of heavy thiols that could be exceedingly difficult to remove from the wine. On the other hand, mercaptans and the other sulphides, are more intractable. Mercaptans impart off-odours reminiscent of rotten onions and disulphides are formed under similar reductive conditions and generate cooked-cabbage odours. Related compounds, such as 2-mercaptoethanol (XV, **Figure 1**) and 4-(methyl thiol) butanol (XVI, **Figure 1**), produce intense barnyard and chive–garlic odours, respectively.

Light-struck refers to a reduced-sulphur odour that can develop in wine during exposure to light [62]. This defect is associated with the formation of volatile sulphur compounds with unpleasant aroma notes, formed by the methionine degradation catalysed by the photochemically activated riboflavin. Methanethiol (XII, **Figure 1**) and dimethyl disulphide (XVII, **Figure 1**) are the main compounds responsible for the light-struck taste in white wine termed as 'cooked cabbage' [82, 83]. Exposure of wine to light at wavelengths close to 370 or 442 nm is particularly effective in inducing the light-struck taste [84], manly when clear glass bottles are used [85]. The preventive strategies are the most efficient as this defect generally develops after wine bottling, and these are mainly related to the reduction of the riboflavin levels in grape juice and wine. There are classic and authorised fining agents, such as bentonite and AC (activated carbon) that can be used to remove with relative efficiency riboflavin from white wine [86]. After application, if bentonite the average residual riboflavin was 60% [86, 87]. Also during the AF, the selection of low riboflavin-producing yeasts can be used as it was shown that it is yeast strain-dependent [86, 87].

Several herbaceous off-odours may be detected in wines. The presence of excessive sensations of herbaceous off-odour results in a decrease in the fruit notes, normally not appreciated by consumers. The source of this off-odour can generally be due to the presence of alkylmethoxypyrazines or aldehydes and alcohols with C6. The main alkylmethoxypyrazines found in grapes, musts, and wines are 3-ethyl-2-methoxypyrazine (ETMP, XVIII, **Figure 1**); 3- sec-butyl-2-methoxypyrazine (SBMP, XIX, **Figure 1**); 3-isopropyl-2-methoxypyrazine (IPMP, XX, **Figure 1**); and 3- isobutyl-2-methoxypyrazine (IBMP, XXI, **Figure 1**), conferring aromatic notes described as 'green pepper', or 'tomato leaf'.

Alkylmethoxypyrazines represent a narrow, delineated group of extremely powerful odorants characterised by extremely low sensory perception thresholds (1–2 ng/L in distilled water [88]; being present in green plant tissues, including grapes [89]. The content of methoxypyrazine in the wine depends primarily on grape composition [90], being observed a complex relationship between viticultural practices and varietal aroma, being difficult to predict the final wine aroma because of the multiple compounds and pathways involved. This vegetative character is most commonly, although not exclusively, associated with Sauvignon Blanc, Cabernet Sauvignon, and other Bordeaux varietals [91]. IPMP may also be present in certain grapes and thus found in the derived wine as a varietal character. The excessive green bell pepper aroma found in red wines containing IBMP is generally considered unfavourable to wine quality. However, the presence of this compound at low levels is often noted to augment the quality of certain wines obtained from red varieties (Cabernet Franc, Cabernet Sauvignon, Carménère, Merlot) or white varieties (Sauvignon Blanc, Sémillon) by adding to the intrinsic flavour complexity of these varietals [92]. The presence of IBMP can be a positive quality factor when it is not dominant but is in balance and complemented by other herbaceous and fruity aromas [93].

Aldehydes and alcohols with 6 carbon atoms are volatile, odorous molecules that can contribute to the herbaceous aroma in the wine. Their cut-grass-like aroma is the characteristic odour of freshly damaged green leaves; therefore, these compounds are often referred to as green leaf volatiles [94] and may also impart a bitter flavour [95]. The C6 alcohols frequently found in grapes include hexanol (XXII, **Figure 1**), (Z)-3-hexenol (XXIII, **Figure 1**), and (E)-2-hexenol (XXIV, **Figure 1**). (E)-2-hexenol, (E)-3-hexenol may also be found in wine at levels of μg/L [96]. The C6 aldehydes commonly identified in grapes are hexanal (XXV, **Figure 1**) and (E)-2-hexenal (XXVI, **Figure 1**); also C7 aldehydes have been found, but at lower content concerning C6 aldehydes [97]. At low levels (< 0.5 mg/L threshold), these C6 volatiles compounds contribute positively to the overall aroma of the wine. These C6 compounds may be present in a free volatile form or in bound form, as glycosides [98]. They are mainly generated through the enzymatic breakdown of C18 polyunsaturated fatty acids contained in plant membranes. The C6 aldehydes and alcohols derive from the oxidation of grape polyunsaturated fatty acids such as oleic acid, linoleic acid, and linolenic acid initiated by the lipoxygenase pathway when the berries are crushed [99]. Their levels in must can be in the order of several hundreds of μg/L [100] or even more than 13,000 μg/L [101], with very variable odour thresholds (400–8000 μg/L) [11]. Their levels depend on several factors, including the grape variety and ripeness, treatments before fermentation, and temperature/duration of contact with the skins.

1,1,6- trimethyl-1,2-dihydronaphthalene (TDN, XXVII, **Figure 1**) exhibits kerosene- and petrol-like off-flavour when present at high levels. Precursors of TDN are carotenoid derived compounds originating from the grapes [102]. These precursors are slowly converted to TDN in the wine acidic medium. Kerosene/petrol aroma usually becomes perceivable after several years of wine storage. TDN is an ambiguous aroma compound, defining the varietal character of Riesling wine but also constituting a repelling taint [103] Comparing wines made of various grape varieties, a perceivable amount of TDN is found mostly in Riesling wines. The recognition threshold of TDN has been reported by Simpson [104] to be in the range of 20 μg/L, while Sacks et al. [105] determined a detection threshold of 2 μg/L. Exposing the grapes to more sunlight by defoliation increases both TDN levels [106]. Low pH and bottle ageing will increase their content likewise due to hydrolytic cleavage of the TDN precursors [102, 106].

3.2 Wine taints

1,8-Cineole (eucalyptol, XXVIII, **Figure 1**) is known to give the perception of eucalyptus and minty flavour, not negative sensory notes by themselves, the reason why there is a discussion if it should be considered as a positive or as a taint in red wine. The sensory perception threshold is very low, of 1.1 μg/L, and a recognition threshold of 3.2 μg/L in Californian Merlot wine [107]. Farina et al. [108] reported similar threshold values in Uruguayan Tannat wine. According to Saliba et al. [109], the mechanism by which 1,8-cineole occurs in the finished wine is not well understood and three mechanisms have been suggested: the compound develops from chemical precursors during the winemaking and bottle ageing processes, namely by chemical transformation of limonene and α-terpineol [108]; that grapes naturally produce the compound during berry development [110]; 1,8-cineole is introduced via another source for example from trees.

Geranium taint is due to the presence of 2-ethoxy-3,5-hexadiene (XXIX, **Figure 1**) in wine, which has an odour reminiscent of crushed geranium leaves. It is originated from the reduction of sorbic acid carried out by the LAB. The reduction product sorbitol under wine conditions isomerises to 3,5-hexadiene-2-ol that after reaction with ethanol generates the 2-ethoxyhexa-3,5-diene which has a sensory threshold of about 0.1 mg/L [111].

Grapevines and grape exposure to smoke from firers can result in wines with undesirable sensory characters, such as 'smoky', 'burnt', 'ashy' or 'medicinal', usually described as 'smoke taint'. Smoke taint markers in grapes and wine are the VPs, guaiacol (XXX, **Figure 1**), and 4-methylguaiacol (XXXI, **Figure 1**) [112]. Kennison et al. [113], showed that trace levels (≤1 μg/L) of guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol were detected in grape juice derived from grapes harvested from grapevines exposed to smoke; but significant quantities of these phenols were released when the grape juice was fermented, or hydrolysed with strong acid or β-glucosidase enzymes. These compounds are known to exhibit 'smoky', 'phenolish', 'sharp', and 'sweet' aromas [113]. Guaiacol causes a phenolic and medicinal taint in a contaminated wine [114], its flavour threshold is 0.030 mg/L in an aqueous solution containing 12% ethanol. An aroma threshold of 0.020 mg/L in a dry white wine was reported by Simpson et al. [115] and a detection threshold in the water of 5.5 μg/L and white wine of 95 μg/L and red wine, 75 μg/L [112].

2,4,6-trichloroanisole (TCA, XXXII, **Figure 1**) is probably the most known compound associated with wine defect, being the key compound responsible for the 'cork taint' in wines [116]. It is very easy to recognise because of its low sensory threshold, which is from 0.03 to 1–2 ng/L in water and 4 ng/L in white wine for trained assessors. However, the threshold values in wine depend strongly on the kind of wine, the wine style, and the experience of the panellist [117]. 'Cork taint' is mostly described as a musty, mouldy, or earthy smell, being sometimes also described as burnt rubber, smoky or even camphor. Other chloroanisoles, such as 2,4-dichloroanisole (2,4-DCA, XXXIII, **Figure 1**), 2,6-dichloroanisole (2,6-DCA, XXXIV, **Figure 1**), 2,3,4,6-tetrachloroanisole (TeCA, XXXV, **Figure 1**) and pentachloroanisole (PCA, XXXVI, **Figure 1**) can also contribute to the 'cork taint' but they do not play a dominant role in this fault. 2,4,6-tribromoanisole (TBA, XVII, **Figure 1**) can also have a significant role in the musty/mouldy of-odour of wines [118]. Moreover, the aroma masking effect of TCA or TBA can be perceived in the wines at levels even lower than perception thresholds (4 ng/L) [118]. As cork is a natural product from the cork oak it is subject to microbial contamination and its quality is dependent on good agricultural practices and quality control during processing, transport, and storage. Chlorophenolic biocides

(nowadays forbidden but accumulated in the environment) are the common precursors which can be transformed by certain fungi to TCA and different chloroanisoles. Other pathways of chloroanisoles formation usually include reactions of chlorination and methylation of compounds naturally present in wooden and cork materials [119]. At the same time 2,4,6-tribromophenol (TBP), which application is not restricted present, can play the role of TBA precursor and increases the risk of musty/mouldy taint in wines [118]. Moulds are considered the most significant causative organisms of cork taint, with implicated genera including *Penicillium*, *Aspergillus*, *Cladosporium*, *Monilia*, *Paecilomyces* and *Trichoderma*. Nevertheless, the process of wine contamination by haloanisoles is complex. Since cork stoppers are the most known source of these compounds, the musty/mouldy fault was named 'cork taint'. However, succeeding studies demonstrated that musty/mouldy defects in wine are not originated exclusively from the naturally contaminated cork materials [118, 120]. TCA and other haloanisoles can be formed in different wooden parts inside the cellar (barrels, ceiling constructions, pallets) and subsequently released into the air. Hereafter, the contamination of winery equipment and 'clean' cork stoppers occurs and haloanisoles can be transmitted further to the wine. Besides, cork taint-related flavours were found in wines that were barrel samples or not closed with natural cork stoppers, indicating that natural cork stoppers are not the only source of mouldy off-flavours [118, 120]. Therefore, depending on the compound causing the cork taint, the consumer has a different impression of the problem. Several compounds with similar negative flavour attributes were discovered in mouldy and musty smelling wines that were not affected by TCA (geosmin, 2-methyl-isoborneol, octane-3-one, pyrazines, etc.) [121]. That said, misguided hygiene practices have historically been part of the cork-taint problem. Cleaning using chlorinated bleach was common in wineries until a link to cork taint was found. Contact between barrels and bleach on cellar floors was a particular pathway for TCA to strike. Flame-retardant paints and fungicides were found to taint wine with TBA. Barrelled wines were particularly badly hit, and some facilities had to be rebuilt. Nowadays most wineries know to avoid chemicals containing tribromophenols. Heat-treated wood is more common, and barrels are rarely cleaned with chlorine. Different approaches were made regarding the removal of TCA and TBA from tainted wines; either by fining with ACs and filtered afterwards, or polyethylene was added as an adsorbent to the wine [121].

Along with TCA and TBA, geosmin (XXXVIII, **Figure 1**), 2-methylisoborneol (MIB XXXIX, **Figure 1**), 1-octen-3-one (XL, **Figure 1**) and 1-octen-3-ol (XLI, **Figure 1**) are compounds that are closely linked with the growth of moulds [116]. Their presence in wines can impart typical earth, mushroom, fungal and mouldy flavour [122, 123]. The mushroom aroma is associated mainly with 1-octen-3-one and 1-octen-3-ol, whereas the earthy aroma is attributed to (−)-geosmin and an earthy-camphor aroma to 2-methylisoborneol. Geosmin may result from the development in grapes picked in unfavourable weather conditions by microorganisms. It is a chiral compound and the (−) form is much more odoriferous than the (+) form. (−) Geosmin is also the only enantiomer to have been identified in pure cultures of *Streptomyces* sp. and *Penicillium* sp. strains isolated from rotten grapes [121]. Geosmin olfactory detection threshold depends on the wine matrix: In water: 10 ng/L [122]; In wine: white wine, 60–65 ng/L [122]; red wine, 80–90 ng/L [122]. MIB is a metabolite of *Botrytis cinerea*, some *Penicillium* spp. and some *Streptomyces* spp. MIB and 1-octen-3-one have also been found in musts made from rotten grapes but not in the corresponding wines, indicating that they are not stable during AF [123]. The findings of both compounds in bottled wine can therefore be linked to the cork stopper and the growing of mould on the cork during the manufacturing process. MIB olfactory detection threshold has been determined as: 0.012 μg/L (in

water La Guerche et al. [123]; 0.04 μg/L [123]; in red wine). 1-octen-3-ol (olfactory detection threshold in the water of 2 μg/L and red wine, 40 μg/L, La Guerche et al. [123], however, has also been found on rotten grapes and the musts made from them and is stable during AF so that an occurrence of this compound in a wine can be caused by mould growth on the grapes as well as by contaminated cork stoppers [124]. Lisanti et al. [125] showed that in the red wine the potassium caseinate and grape seed oil treatments decreased the level of geosmin by 14% and 83%, respectively, while in the white wine, the AC and the grape seed oil were able to decrease the level of geosmin by 23% and 81%, respectively. However, after estimating the olfactory impact of the volatile compounds by OAVs (concentration/odour perception threshold), only the treatment with grape seed oil was able to decrease the relative contribution of geosmin in the profile of the odour active compounds, in both wines.

Wine can accidentally be contaminated with styrene when trace amounts of the styrene (XLII, **Figure 1**) are released during wine storage in polyester tanks reinforced with fibre glass [126]. Also, occasionally styrene contamination has been detected in wine in contact with synthetic closures [127]. The taste threshold for styrene in water has been reported as 22 μg/L [128] but may be higher in wine. An amount higher than 100 μg/L (the generally accepted threshold of sensory perception), styrene can modify the wine sensory characteristics by imparting a taste of plastic and adhesive. Wagner et al. [129] found in German wines values ranging from 0 to 19 μg/L.

4. Origin of taste and tactile sensory defects and strategies for wine stabilisation

The wine imbalances by acidity, astringency, or bitterness, are often the first defects noted in the sensory perception of wine quality [13].

4.1 Acidity

Organic acids are the main responsible for sourness and able of modifying this sourness sensation in wines producing a pleasant and refreshing sensation [130]. However, when present at high levels they are responsible for an unpleasant acidity. Therefore, it is generally accepted that too much acidity will taste excessively sour and sharp, while wines with too little acidity will taste flabby and flat and present a less defined flavour profile [131]. Organic acids contribute to the tartness and mouth-feel properties of wine. Tartaric acid is the main organic acid in wine, which, at high levels (>5 g/L), is responsible for an unpleasant taste. Other acids include malic, citric, fumaric, succinic, pyruvic, α-ketoglutaric, lactic, and acetic [3]. However, different organic acids have different sensory properties, and the impact of organic acids is therefore not only linked to total acidity and pH, but to the specific levels of each acid in the wine [132]. The perceived sourness was imparted by L-tartaric acid, D-galacturonic acid, acetic acid, succinic acid, L-malic acid, and L-lactic acid and was slightly suppressed by the levels of chlorides of potassium, magnesium, and ammonium [16]. Acidity adjustment is the reduction or increase in titratable acidity so that the resulting wine will be acceptable. Acidity adjustment can be performed by the addition of an approved acid, the chemical deacidification with approved salts, and using ion exchange resins, either cation, anion or both, electromembrane processes and by biological deacidification. Tartaric acid is commonly used to increase the titratable acidity and reduce the

pH in the wine industry, because of its stability and the fact that yeast and other microorganisms are unable to metabolise it at wine pH [133].

The reduction of titratable acidity by the addition of carbonate salts such as calcium carbonate, can be done in one of two ways, the first, is a direct addition which is not recommended as it results in wines which are unstable with respect to calcium tartrate, the second is to treat only a wine portion. This process causes the pH to increase up increase to 4 or 4.5 at the end of the addition. The tartaric and malic acids are primarily in the ionised forms. The precipitation of their calcium salts is favoured and this also lowers the calcium levels. This method is referred to as the double-salt and it may be used to reduce the total acidity of high-acid grape musts before fermentation. The precipitation is primarily that of calcium tartrate and under certain circumstances the coprecipitation of calcium malate [2]. Ion exchange resins, either cation exchange alone or as a combination of anion and cation exchange, can also be used to change wine acidity [19]. In red wine for example the acid reduction can be achieved by using LAB strains. MLF refers to the conversion of malic acid to lactic acid and CO_2. This secondary fermentation usually takes place after the AF. The benefits of MLF is the acidity reduction and simultaneously add the complexity of aroma and taste and provides a more microbiological stable wine [2].

4.2 Astringency

One of the most important sensations and a quality attribute is astringency. Gawel et al. [134] presented a structured vocabulary derived by a panel of experienced wine tasters that describe the astringent sub-qualities of red wines, such as velvety, drying, puckering, or roughing. Astringency is mainly a tactile sensation [135] not a taste because it can be perceived in regions of the oral cavity where there is no taste receptor [96, 136, 137]. The major mechanism proposed to astringency perception is the interaction and precipitation of salivary glycoproteins, namely by tannins generating a loss of lubrication [136]. Vidal et al. [138] showed in model solutions that astringency perception of proanthocyanidins increases with their mean degree of polymerisation (mDP) and their percentage of galloylation [139]. Oligomeric proanthocyanidins have been described as inducing lower roughness than the more polymerised molecules, whereas an increase in galloylation has been associated with a higher perceived drying and roughing astringency [139]. However, other wine phenolic compounds, such as flavonols, phenolic acids, or anthocyanins, can also play an important role in astringency development [139].

4.3 Bitterness

Bitter perception in wines is related to phenolic compounds with low molecular weights as well as to monomeric or small phenolic flavanols [16]. Concerning the latter, they have been described for a long time as the main contributors to the bitterness generated by flavonoid phenols [140]. Monomeric flavonoid phenols are primarily bitter but as the molecular weight increases upon polymerisation, astringency increases more rapidly than bitterness. It has also been shown that chiral difference between the two major wine monomeric flavanols produces a significant difference in temporal perception of bitterness: (−)-epicatechin is significantly bitterer and has a significantly longer duration of bitterness in the mouth than (+)-catechin [140].

Protein fining agents could induce some sensory changes. Astringency and bitterness of wine can decline due to its interaction with tannins. The fining process

directly occurs from the precipitation of proanthocyanidins by these protein fining agents and it is influenced by the chemical characteristics of the protein used. The interactions between proanthocyanidins and protein fining agents depend on molecular weight, amino acid composition and surface charge density of the proteins used [141–144].

Different proteins are used for wine fining such as gelatine, egg albumin, isinglass, and casein/potassium caseinate. Different types of gelatine remove different amounts of proanthocyanidins (9–16%) depending on the wine phenolic composition and structural characteristics of the proanthocyanidins and on the gelatine composition and characteristics [142, 143]. It has been generally thought that proteins bind primarily high polymerised tannins as well as high galloylated tannins, and therefore are preferentially removed [141], but some recent work showed that each of the different proteins (gelatine, egg albumin, isinglass, casein) and different size fractions of the same protein class interact differentially with different sizes of tannins [142, 143]. Regardless, allergen labelling may make wine fining with any of the animal-derived products impractical although some effort has been made to evaluate plant-derived proteins [144]. Recent studies of wine astringency demonstrated that tannins must be different two-fold for a trained panel to be able to successfully differentiate the wines [145]. Further, since some of the polymeric pigments can precipitate with protein there is the risk of losing stable colour [146]. As mentioned previously a higher astringency intensity is directly associated with a higher concentration of proanthocyanidins with a higher mean degree of polymerisation [147]. During ageing, astringency perception becomes softer, the reasons for the change in wine astringency could involve a decrease in proanthocyanidin concentration accompanied by a decrease in proanthocyanidins structural changes [148].

Therefore, the phenolic composition could be modulated during the winemaking steps (maceration/fermentation, stabilisation (fining) and ageing) and consequently, it allows the modulation of wine astringency and/or astringency sub-qualities as well as the wine bitterness.

5. Origin of potentially toxic compounds and strategies to improve wine safety

In fermented beverages in which a variety of microorganisms exist it may be inevitably the production of toxic products as a result of their metabolism and side reactions, including ethyl carbamate (I, **Figure 2**), biogenic amines (II, **Figure 2**) mycotoxins, namely ochratoxin A (III, **Figure 2**) and aflatoxin B1 (IV, **Figure 2**). They are generally generated due to the incomplete metabolism of nitrogen-containing compounds during the fermentation process [149].

5.1 Ethyl carbamate

Ethyl carbamate (EC), also known as urethane, is an ethyl ester of carbamic acid that can be found in several fermented beverages [150] including wines [151]. EC levels in wines can range from n.d.-19 μg/L in white wine, n.d.-54 μg/L in red wine, 14–50 μg/L in fortified wine, and n.d.-58 μg/L in sherry-typewine [152]. EC is classified as a 'probable human carcinogen' by the IARC since 2007 (group 2A) [153]. Although currently there is no harmonised maximum level for EC, some countries have established their criteria for example in Canada the maximum level is 30 μg/L for wine, the Canadian guidelines were adopted by other countries such as Czech Republic, Brazil, France, Germany, and Switzerland. South Korea also set the maximum limit of 30 μg/L only for table wine. For fortified wine, the maximum level of

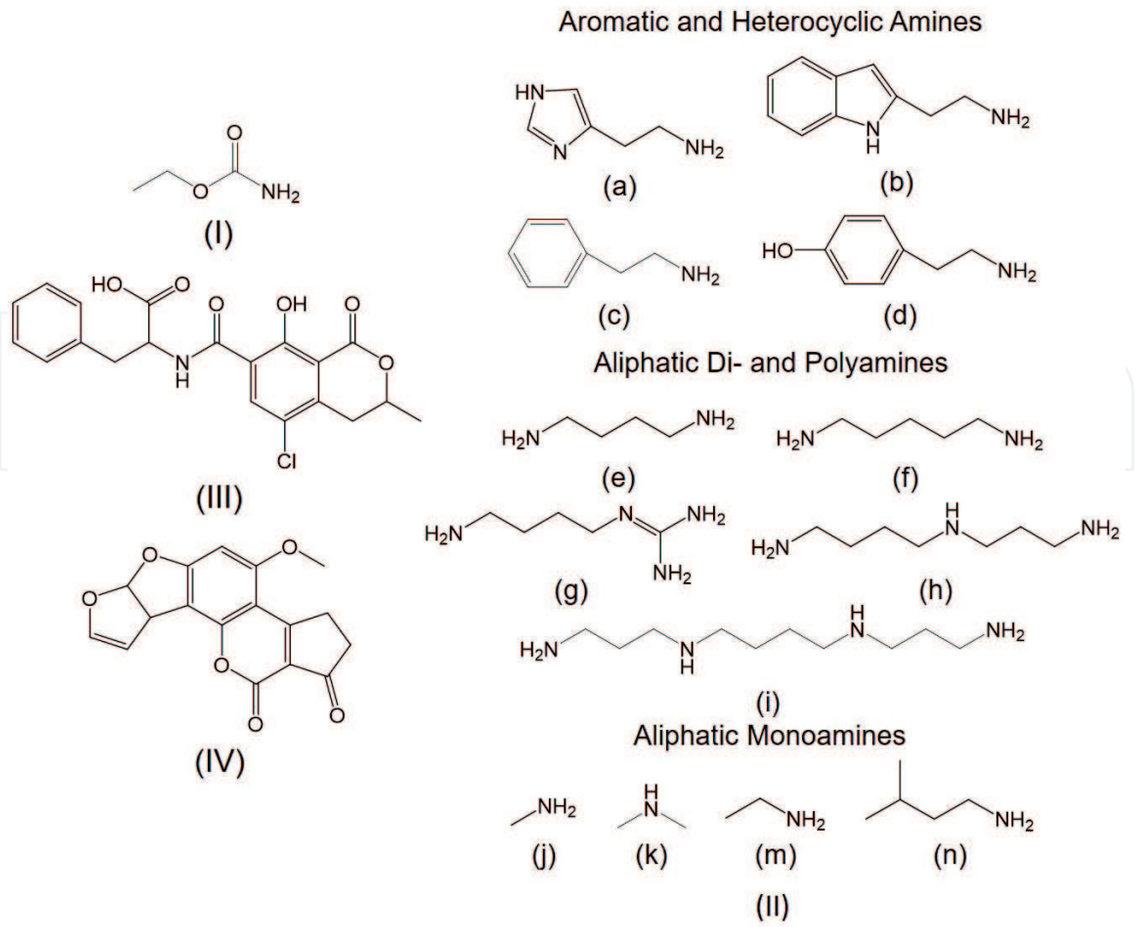

Figure 2.
Main wine potentially toxic compounds. (I) EC; (II) BAs – (a) histamine; (b) tryptamine; (c) phenylethylamine; (d) tyramine; (e) putrescine; (f) cadaverine; (g) agmatine; (h) spermine; (i) spermidine; (j) methylamine; (k) dimethylamine; (m) ethylamine; (i) isopentylamine; (III) OTA; (IV) AB1.

EC is 100 μg/L in Canada and the Czech Republic, and of 60 μg/L in the US [154]. EC can be produced from at least five precursors, namely urea, citrulline, carbamyl phosphate, cyanic acid and diethyl pyrocarbonate. In turn, urea and citrulline can be respectively generated by yeast and LAB by metabolisation of arginine, a major amino acid found in grape juice and wine [155]. The fermentation conditions, such as pH, temperature, ethanol level, light irradiation, oxygen, storage time, yeast or LAB strains can also affect the formation of EC [156]. For example, lowering the temperature during fermentation and storage, lowering the pH, lowering the ethanol content, and addition of diammonium phosphate as a yeast nutritional supplement reduces the EC concentration. The development of techniques for EC elimination from alcoholic beverages [155] has attracted considerable attention, and enzymatic decomposition methods have been widely employed given their safety and environmentally friendly nature. Two enzymes are used, namely, urease, which is commercially available and can degrade urea, the major precursor of EC [157], and urethanase, which can directly catalyse EC degradation [158]. To reduce EC concentration in wine, the use of acid urease seems to be the most appropriate way to suppress EC formation [19, 159]. Moreover, the efficiency of commercial acid urease treatment varies with several factors, including pH, temperature, the presence, and concentration of inhibitors (malate, ethanol, phenolic compounds), and type of wine [157]. Therefore, the immobilisation of acid urease in chitosan beads enhances the protection against inhibitors, increases the stability of the enzyme, and has the advantage of facilitating enzyme recycling and consequently reducing the cost of its use. EC content can also be effectively reduced by decreasing the

generation of its precursors. Significant advances have been made via genetic technologies in modifying fermentation strains that produce less EC precursors. Genetic modification approaches have the potential to provide safe, affordable, and effective methods to decrease EC formation. Several studies have shown that the modification of catalytic enzymes, such as urea carboxylase, arginase, and allophanate hydrolase, showed the ability to reduce the concentration of EC [156]. Additionally, the modification of urea permease and amino acid permease, which are regulated by several factors and directly affect the generation of EC precursors have been explored [160]. Since the metabolism pathways related to urea have been fully considered for the high-efficiency minimisation of EC, enhancing the gene expression of $DUR_{1,2}$ and DUR_3, which encode urea degradation enzymes and permease, respectively, is considered to be a viable strategy. In this way, the modification of permease has led to the construction of functionally enhanced urea-importing wine yeast cells, which can continuously express the DUR_3 gene and reduce EC level in Chardonnay wine by 81% [161].

5.2 Biogenic amines

Biogenic amines (BAs) are low molecular weight organic bases that have adverse physiological effects on humans when absorbed at high levels [162]. BAs are formed by decarboxylation of the corresponding amino acids by microorganisms such as LAB [162]. *Pediococcus,* as well as *Lactobacillus,* have been implicated in the production of BAs in wines that have undergone spontaneous MLF [162]. The final BAs levels in wine depend on the availability of the precursor amino acids and the BAs producing bacteria [162]. The only currently available simple and efficient solution to avoid or minimise BAs formation in wine is the use of MLF starter cultures [163]. After inoculation, the selected strain becomes dominant during MLF. Another recommendation is to avoid the practices that increase amino acid and peptide levels in musts. This also implies that LAB have more substrates, not only for producing BAs but also to survive better and longer after MLF [164]. Although there are currently no official values for the maximum limits for histamine and other BAs, the maximum value imposed for the levels of histamine, has been established through wine purchase and sale contracts, with the German companies demanding a maximum level of 2 mg/L of histamine. For these reasons, strategies for the reduction/elimination of BAs in wine are necessary, especially histamine. Until now, there are not useful treatments for reducing BAs levels especially, in red wines. However, it has been shown that uncommon wine LAB strains have amine oxidase activities that degrade histamine, tyrosine, and putrescine [165]. Also, non-*Saccharomyces* yeasts, such as *Schizosaccharomyces*, can decrease malic acid content in wine, they can be an excellent alternative to LAB, avoiding the MLF. Also, the use of *Schizosaccharomyces* reduces the risk of BAs production [166]. According to the OIV Resolution [19], only bentonite is applied in already contaminated wines to reduce the content of the BAs in the final wine [167]. Bentonite has a negative surface charge density, being able to exchange the cations adsorbed on its surface by the wine BAs. However, due to the negative impact on wine aroma combined with the high wine losses due to the high volume of lees, bentonite is presently not an adequate solution and other options need to be studied. Till now, there is no effective way of removing BAs in the finished wine.

5.3 Mycotoxins

Mycotoxins are secondary metabolites produced by several fungi that grow in food, including wine, under particular circumstances, with ochratoxin A (OTA) being one of the most important [168]. In 1993, the International Agency for

Research on Cancer (IARC) classified OTA as possibly carcinogenic for humans (group 2B) [169]. Since 2006, the maximum limit for OTA in wine is 2 μg/kg [170]. OTA has been related to wine contaminations since 1996 [171] and after that, the occurrence of OTA in wine samples has been described in several works. Blesa et al. [172] found an OTA incidence in wines of 53% in 521 red wines, 69% in 98 rosé wines, and 61% in 301 white wines. These data show that it is important to prevent and control the occurrence of this mycotoxin in wines. To eliminate this toxin, several chemicals, microbiological and physical methods have been described [168]. Nevertheless, in the case of wines, effective removal processes are limited, and at present, the use of adsorbents is the most common. OTA content can reduce in the final wine from 70 to 32% by fermenting with selected yeast [173], *Non-Saccharomyces*, such as *Schizosaccharomyces*, are also promising in reducing the OTA content by about 70% during fermentation [173]. Several fining agents have been evaluated concerning their ability to remove OTA from wines, and it was found that AC presented a good adsorption capacity for OTA [174]. Filtration before bottling about 0.45 μm of wine can easily reduce the final content in OTA by about 80% [174], but none of them is 100% efficient in removing OTA from wines.

Aflatoxins are a group of highly toxic secondary metabolites produced by fungi of the genus *Aspergillus* [175]. AFB1 is the most predominant and toxic aflatoxin. It is classified as a Group 1 human carcinogen (IARC) [169]. Aflatoxins and Aflatoxins-producing strains [176] have been detected in grape and musts [177]. The presence of aAFB1 in wines is caused by fungi that grow on grapes in the vineyards. In the literature, there are few studies regarding aflatoxins contamination in wines [177]. One of them is from Di Stefano et al. [178] that studied the occurrence of aflatoxins in 30 sweet wines from five winemaking in the Sicilian regions, Italy. The presence of aflatoxin in wines has been documented in recent years, largely because of the adaptability of aflatoxigenic fungi, such as *A. flavus*. At present, the EU has not set a maximum allowable limit for aflatoxins in wine, but this does not mean that the problem can be ignored. Therefore, it is essential to develop technological solutions to reduce/eliminate the levels of aflatoxins in wines. Recently the work from Cosme et al. [179] shows the high efficiency of bentonite in the removal of aflatoxin B1 and B2.

6. Conclusions

As overviewed in this chapter, there are many physicochemical wine instabilities and defects that can appear during wine production. Some defects can significantly decrease the wine's sensory quality. However, today, there are several treatments and solutions to avoid them or reduce their impact on wine quality. The best strategy is always a preventive approach. Nevertheless, some of the defects are intrinsic to the grape composition and/or wine production process therefore they must be removed or minimised before bottling. The available treatments, either by using fining agents, additives, or other technological solutions, are generally effective, although they are sometimes not the perfect solution as they can also impact on positive wine sensory attributes and/or have a detrimental environmental impact. Current research trend is focused on the development of fining agents, additives, or technological solutions with improved specificity that will allow the removal of the defect without changing the other wine characteristics, and at the same time to explore low cost, natural or renewable materials that will allow a lower environmental impact of the stabilisation process.

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