Chapter 5

Contribution of Yeast in Wine Aroma and Flavour

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Additional information is available at the end of the chapter

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Abstract

Organoleptic characteristics of wine, especially, the spectrum that is defined as flavour and aroma, are the most important parameters for assessing the quality of wine. The origin of these characteristics comes for four main sources: grapes, vinification, maturation and ageing. The final concentrations of various odour-active components (OAC) are highly depended on the yeast during fermentation. The major OAC that are formed during fermentation are volatile substances like esters, higher alcohols and carbonyl compounds. Decoding the origin and contribution of these OAC, the modern winemaker can direct and manipulate the yeast during fermentation on his benefit. These compounds are originated from the secondary metabolism of the yeast, understanding the role of the key parameters during fermentation influencing the OAC formation like temperature, yeast assimilable nitrogen (YAN) and suspended solids is vital for the final organoleptic characteristics of wine.

Keywords: yeast, wine, aroma, flavour, fermentation, volatiles, esters, higher alcohols

1. Introduction

Wine is the alcoholic beverage which is the product of fermentation, usually, of fresh grape must. Wine consists mainly of 86.8% water and weight by volume concentration of the following: 11.2% ethanol, 0.5% acids (volatile and non-volatile), 1% trace components (sugars, anions, cations, etc.) and only a very small portion of 0.5% of volatiles contributing to the aroma of wine [1, 2], often described as odour-active compounds (OAC). These compounds are part of the olfactory fingerprint of each wine. The concentration and the ratio between various groups of OAC are unique not only to each wine but also to each terroir, to the style of vinification and maturation procedures. Yeast has a major role in the wine aroma formation and modulation, apart from the formation of alcohol. According to Fleet [3], the way that yeast influences the final aroma of wine can follow these six mechanisms:

i. Involve in the biocontrol of moulds on grape, which influences quality before harvest.

ii. Perform alcoholic fermentation of must sugars and transform juice into wine; the *de novo* biosynthesis of the flavour and aroma compounds.

iii. Enzymatic conversion of flavour neutral, grape components into odour-active compounds.

iv. Alterations of OAC profile through the yeast autolysis products.

v. Absorption of grape juice components.

vi. Spoilage of bulk wine throughout the storage period and even after packaging.

vii. Influence growth of other spoilage microorganism, for example, lactic acid bacteria, acetic acid bacteria.

The single most important mechanism, which can be manipulated by the winemaker, of the above list is that of fermentation. Due to the fact that during the procedure of fermentation the largest concentration the final OAC in wine is formed [3–5]. The input and manipulation of winemaker that can influence the final product, this is done through decision-making for the implementation of various vinifications practises and, like the fermentation temperature [6–10], inoculation [11–13], addition of yeast assimilable nitrogen (YAN) [14–17] and the initial total concentration of the suspended solids of must before inoculation [18–21].

Throughout the fermentation process, the environment, which the yeast is called to function, is under continuous changes [22]. During fermentation, a vast amount of heat is being produce by the yeast, although this is counter balanced, and is easily control, by the modern temperature control tanks; temperature is one of the main limiting factors, for yeast growth. Also due to the sugar transformation and the utilisation of oxygen and YAN, yeast should engage various mechanisms in a diverse unaffordable growth conditions, called 'stress', to the emerging environment alterations [22]:

i. Osmotic pressure alterations

ii. Limitation of essential nutrients

iii. Ethanol toxicity

iv. Production of by-products toxic to the cell

The continuous need for adaptation to this kind of environment emerges the need for the corresponding adaptation on various responses, in order to maintain the intracellular metabolic activity [22].

Fermentation of sugars by yeast can be divided into two stages: the primary and the secondary metabolism. By primary, we mean the metabolism that is essential for yeast growth and cell division, producing compounds like ethanol, glycerol, acetaldehyde and acetic acid. Secondary metabolism is non-essential for growth and produces small molecules. Through this secondary metabolism, yeast adaptation procedures can mainly influence the final wine aroma profile [23]. Namely, the FAC generating mechanisms in which the yeast is engaged are the following [3]:

i. Utilising grape juice constituents
ii. Producing ethanol and other solvents that help to extract flavour components from grape solids
iii. Producing enzymes that transform neutral grape compounds into flavour active compounds
iv. Producing many hundreds of flavour active, secondary metabolites (e.g. acids, alcohols, esters, polyols, aldehydes, ketones and volatile sulphur compounds)
v. Autolytic degradation of dead yeast cells

2. Wine aroma: origin and type

Overall, the aroma of wine can be distinct into primary, being the OAC derived directly from the fruit; characteristic to the grape variety, secondary aroma generated during the vinification/fermentation and lastly maturation and ageing procedures are responsible for the group of aroma characteristics that are described as tertiary [24, 25].

The major OAC are divided into four big groups: esters, aldehydes, alcohols and terpenes [25, 26]. In addition to these, there are also two other groups that are very characteristic for specific grape varieties, these are pyrazines (primary aroma) and sulphur compounds like polyfunctional sulphur compounds (4-mercapto-4-methyl-pentan-2-one, 3-mercaptophenol) and dimethyl sulphide DMS [27]. Each one of these groups plays a unique role in the perception of the aroma character of the final wine. Also all these groups have a diverse formation pathway.

2.1. Flavour active groups contributing in wine aroma

2.1.1. Esters formation and contribution

Esters are the group with the highest importance in wine and are usually the most predominant in the formation of the flavour character of the final product [22]. Esters are formed with the combination of alcohols and organic acid with the elimination of water [25]. In wine, two types of this group can be met: first, the one that is modulated enzymatically by the yeast enzyme pool and second, the one that is formed during ageing [2, 28]. The enzymatic biosynthesis of ester is catalysed mainly by two types of enzymes: esterases and lipases. The final profile of esters in wine depends on various parameters, many authors pointed the variety and quantities of esters, in **Table 1**, are the main esters found in commercial wine [29]. The group of ethyl acetate, isoamyl acetate, isobutyl acetate, ethyl caproate and 2-phenyl acetate are descripted as the most important esters affecting wine flavour [28, 30, 31].

The net concentration of ester in wine at any given time varies due to the fact that wine is a fairly complex matrix substrate, with a number of different compounds involve in various procedures [2]. This depends on the enzymatic activities of synthesis and ester hydrolysis. Maximum concentrations of esters during fermentation observe around 9–12% of ethanol [8, 32].

Compound	Sweet wines (mg/l)	Dry wines (mg/l)
Ethyl acetate	96.56 ± 39.75	85.00 ± 12.54
Isobutyl acetate	0.07 ± 0.02	0.07 ± 0.04
Ethyl butyrate	0.31 ± 0.09	0.41 ± 0.05
Isoamyl acetate	1.81 ± 0.91	2.37 ± 0.62
Ethyl hexanoate	0.87 ± 0.41	1.06 ± 0.19
Hexyl acetate	0.06 ± 0.04	0.14 ± 0.14
Ethyl lactate	13.5 ± 6.6	23.00 ± 18.88
Ethyl octanoate	1.57 ± 0.73	2.11 ± 0.49
Ethyl decanoate	0.65 ± 0.26	0.56 ± 0.06
Benzyl acetate	0.004 ± 0.004	0.003 ± 0.001
2-Phenylethyl acetate	0.23 ± 0.17	0.21 ± 0.05
Ethyl dodecanoate	0.079 ± 0.053	0.021 ± 0.007

Table 1. Range of ester contents in commercial white wines [29].

Esters formed during alcoholic fermentation, by enzymes; fall into two main categories. The ethyl esters of organic acids and the acetates of higher alcohols [23]. The ethyl esters comprise of an alcohol group (ethanol) and an acid group (small, medium-chain fatty acid) (**Figure 1**). The acetate esters are comprised of an acid group (acetate) and an alcohol group which is either ethanol or a higher alcohol derived from amino acid metabolism (**Figure 2**). The latter are responsible for the pleasant fruity aroma of wines [2].

Formation of these two groups of esters during alcoholic fermentation involves a series of various proteins and genes. Today six genes have been identified, with their corresponding protein, to be involved in either the synthesis or hydrolysis of esters in yeast cells. Namely, these are ATF1, Lg-ATF1, ATF2, EHT1, EEB1 and IAH1 [2, 5]. The first three are involved in the mechanism of alcohol acetyltransferase with the first to be the most studied and important in the total quantity of esters formed. EHT1 is involved in the ethanol hexanol-transferase mechanism for the synthesis and hydrolysis of medium-chain fatty acids ethyl esters. EEB1 is

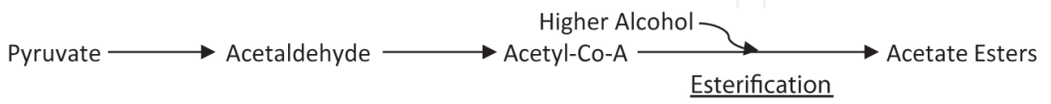

Figure 1. Formation of acetate esters by the esterification of acetyl-Co-A with a higher alcohol [23].

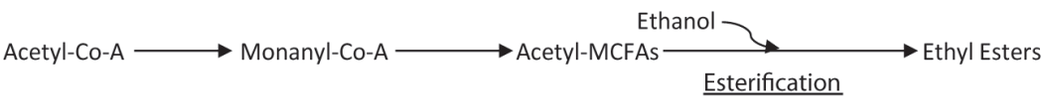

Figure 2. Formation of ethyl esters by the esterification of medium-chain fatty acid (MCFA) with a ethanol [23].

involved in the ethanol acyltrasferase and ethyl hydrolase mechanism. Lastly, the IAH1 is a gene involved in the mechanism of esterase, for the hydrolysis of acetate esters [2, 5].

The acetate ester synthesis is an energy requiring mechanism [31], the reasons why esters are formed during the fermentation procedure are not quite clear. One approach to this is that the synthesis is associated with the detoxification effect by the removal of fatty acids [31]. Even though the transfer of esters through the membrane is directly associated with the length of their chain that is varying from 100% small medium-chain to 8–17% for a long chain (ethyl decanoate) [2]. Another systematic approach for the formation is that of the maintenance in the balance of the acetyl-CoA and CoA-SH pool. It is proposed that these are formed as over-spill products from the fermentation through the sugar metabolism [31].

2.2. Higher alcohols or fusel alcohols origin and contribution

Higher alcohols refers to the group of alcohols that have more than two atoms of carbon on their molecule. This group of compounds with esters are the two biggest groups contributing to the aroma of wine. The vital step in the synthesis of these compounds is the formation of α-ketoacid [33]. Based on the origin of α-ketoacid fusel alcohols can be divided into two categories. The first has origin of α-ketoacid the amino acids and the second, the anabolic pathway of sugars [27]. From the first group, a list of higher alcohols is shown in **Table 2**, were as from the former 1-butanol and 1-pentanol are formed. Their contribution to wine aroma is consider positive when the concentration of these compounds is up to 300 mg/l, above this level the pungent odour is profound [25, 31]. The utilisation of nitrogen sources is strongly associated with the biosynthesis of these higher alcohols [17, 34, 35]. The nitrogen composition and nature (organic or mineral), of the must, are influencing the biosynthesis of these volatiles. It has been shown by many studies that the initial concentration and type of amino acids in the must in some cases is strongly associated with varietal aromas [5]. The observation that the increase in the concentration of certain amino acids led to the increase of the production of specific fusel alcohols let to the formulation of the Ehrlich pathway [33]. Also the well documented Ehrlich pathway intermediates can be found in bibliography, stating, the following **Table 2** of the 'substrate' amino acid and their corresponding fusel alcohol [5].

Amino acid	Higher alcohol
Leusine—Leu	3-Methylbutanol
Valine—Val	2-Methylpropanol
Isoleucine—Ile	2-Methylbutanol
Phenylalanine—Phe	2-Phenylethanol
Tyrosine—Tyr	2-(4-Hydroxylphenyl) ethanol
Tryptophan—Trp	2-(Indol-3-yl) ethanol
Methionine—Met	3-(methyl thio) propanol

Table 2. Flavour-producing amino acid catabolism via the Ehrlich pathway [5].

The biosynthesis in the Ehrlich pathway starts with the transamination of the amino acid producing a α-ketoacid [33]. Followed by the decarboxylation α-ketoacid to a fusel aldehydes [33]. Finally is the decisive step were the fusel aldehyde is reduced to fusel alcohol or oxidised to the corresponding fusel acid. This step is highly dependable on the growing condition during the utilisation of amino acids [27, 33]. In aerobic conditions, amino acids are converted predominantly to fusel acids where as in anaerobic the product of the pathway in almost entirely fusel alcohol. However, the procedure is far from simple, since a vast number of genes and their corresponding proteins are involve in every stage.

Four *S. cerevisiae* proteins have been implicated in the initial transamination step of the Ehrlich pathway (**Figure 3**). Twt1p (also known as Bat1p or Eca39p) is the mitochondrial branched-chain amino acid aminotransferase, and Twt2p (Bat2p or Eca40p) is the cytosolic isozyme. The mitochondrial isozyme is highly expressed in batch cultures during exponential growth and is repressed during stationary phase, while the cytosolic isozyme has the opposite expression pattern [33].

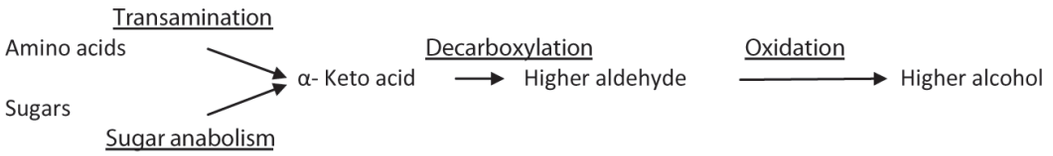

Figure 3. Formation of higher alcohols by the Ehrlich pathway and the anabolism of sugars.

The course of the formation of various higher alcohols was the study of different researchers. According to Fraile et al. [14, 36], the formation of different alcohols takes place at the end of the fermentation, when most of the amino acids have been consumed, whereas, according to Rapp and Versini [37], this synthesis occurs at the same time as ethanol production. A more recent study by Hernandez-Orte et al. [38] monitoring the course of formation of alcohols, among other volatiles, clearly shows that the formation of isoamyl, isobutanol and β-phenylethanol are generated throughout the entire alcoholic fermentation.

2.3. Carbonyl *c*ompounds origin and contribution

The two major compounds in this group are acetaldehyde and diacetyl. Acetaldehyde, which is one of the main metabolic intermediates in alcoholic fermentation, is the last precursor in the anaerobic pathway before ethanol. The pyruvate, end product of glycolysis, is converted to acetaldehyde by the pyruate decarboxylase enzymes, which is further converted to ethanol, by the dehydrogenase enzymes. Another source of acetaldehyde is the oxidation of ethanol during ageing or the activity of film forming yeast to the wine [25, 27] 'flor' effect.

Diacetyl is formed in small quantities in wine by the yeast. This can further metabolised to the corresponding end product of 2,3-butanediol or the intermediate, acetoin. Concentrations of up to 100 mg/L of acetaldehyde and 1–4 mg/L of diacetyl can be described as desirable and that these are contributing to the complexity of the aroma of wine [25, 27].

2.4. Thiols contribution and origin

Thiols are the group of alcohol compounds that oxygen was replaced by sulphur in the hydroxyl group. This compound group is very characteristic for wine aroma, especially for the Sauvignon blanc variety, and is mostly depended on yeast metabolism. The three thiols that have been identified in wine are 3-mercaptohexan-1-ol (3MH), 3-mercaptohexyl acetate (3MHA) and 4-mercapto-4-methylpentan-2-one (4MMP). These compounds apart from been characteristic are also very interesting for studying, exhibiting very low perception thresholds, that is, for 4MMP 3 ng/L, for 3MH 50–60 ng/L and for the acetylated form of 3MH the 3MHA is down to 2–4 ng/L [39]. In addition, the 3MH and 3MHA both have two enantiomers, as chiral molecules, R and S. The aroma the S-3MH and S-3MHA forms were described as passion fruit whereas the form R-3MH as grape fruit and R-3MHA as box tree [40].

Over the years, on Sauvignon blanc variety, two different molecule classes have been proposed as precursors of thiols, the amino acid-based compounds [41, 42] and the non-amino acid [43]. Most studies are focused on the first class of compounds and more specific to Cysteine-conjugates and Glutathione-conjugates. Interestingly, the amino compounds not only are the main precursors but also there was the evidence that glutathionylated precursors can be converted into cysteinylated precursors [44]. The non-amino acid precursor compounds were suggested to be the mesityl oxide and E-2-hexenal [43].

2.5. Terpenes: de novo synthesis and/or biotransformations by yeasts

Clearly terpenes are responsible for some of the most prominent, characteristic and important aromas in grapes and wines. It has been documented early on (1978) that beside grapes, yeasts are also capable of producing terpenes (citronellol, linalool and geraniol by *Kluyveromyces lactis* [45]. Enzymatic activity by yeasts is also possible in relation to liberation of terpenes from sugar molecules and β-glucosidase is well documented [46].

Saccharomyces cerevisiae shows some enzyme activity in different strains [47] but most studies demonstrate significantly higher enzyme production from non-*Saccharomyces* species [48–51]. Efforts to identify the most efficient non-*Saccharomyces* are showing the potential these yeasts have in modern wine making and mix cultures. *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* are enhancing a very good aromatic profile if used in combination with *S. cerevisiae* [52–55]. Efforts to further our knowledge on the related pathways [56] as well as better exploiting the capacity of mixed cultures (*Saccharomyces* with non-*Saccharomyces*) are copious [57–62].

3. Fermentation conditions and influence to wine aroma

The winemaker through the process of vinifications has various parameters that can use in his/her benefit, in order to manipulate the outcome of the process. These parameters are the

temperature and molecular oxygen availability during fermentation, maturation and ageing, the nitrogen source, for the growth and propagation of yeast, inoculation size and yeast strain of the starting culture [3, 31], as well as the nature and quantity of the solids derived from the grapes. All these play a decisive role in the vinification strategy and style that the winemaker wants to follow. Although for most of the OAC, the formation pathway and production promoting parameters are clear in some cases the knowledge behind synergies between these parameters are not quite apparent. The winemaker also has to deal with the changing environment of the fermentation, and more specific to the metabolism of sugar into ethanol and carbon dioxide [9, 22]. These two metabolites, but more essentially, ethanol build-up consecration plays a significant role for the physiology of the yeast. First, high concentrations of ethanol are related to the reduced water activity; this has a triggering effect to the production of various compounds to counter-balance this, but most importantly is the functional alteration of cell membrane that is influencing the uptake of various essential nutrients, important for the yeast survival and growth, including nitrogen compounds, YAN [9, 22]. Although fermentation is a well-known anaerobic pathway, carbon dioxide concentration also is influencing indirectly, the availability of proline utilisation. Specifically, the saturation of must with CO_2 is having as a consequence the elimination of dissolve molecular oxygen, which is needed by oxidase for the first step in proline degradation. This is precluding the utilisation of proline, which is the main amino acid in grape must.

3.1. Temperature effect on aroma formation

Temperature conditions are associated with all enzymatic reactions rate so forth the metabolism and growth of yeast among other microorganism. The temperature range between 15 and 25°C, during wine fermentation is considered favourable for yeast growth under winemaking conditions. The fact that aromatic profile can be modulated during fermentation was noticed very early, since temperature not only affects the volatile composition but also in the case of red wines the extraction of phenolic compounds from the skin and grape seeds [7, 10]. The most noticeable and well-known effect, to winemakers, of temperature is on the fermentation rate and completion which is defined by the total consumption of sugars. Fermentation at 28°C compared to one at 15°C was observed to be 2.5 times faster [7]. Temperature is influencing not only the production of FAC but also the concentration of primary metabolites like ethanol and glycerol, on which it seems temperature to have a reverse effect. In low temperature, the production of ethanol is counter to the glycerol production [7, 9]. From very early studies it was pointed out that the final concentration of esters, contributing to the fruity flavour of the wine, was favoured by low temperatures during fermentation [8]. Particularly esters associated with pleasant fruity aroma, like isoamyl acetate and n-hexyl-acetate, accumulated in higher consecrations at low temperatures. Whereas in high temperature fermentation higher accumulation of ester characterised as heavy odorants like ethyl-octanoate and ethyl decanoate, was observed. Higher final consecration of 2-phenylethyl-acetate was favoured in higher temperatures, by some authors is consider pleasant with rose like odour [6–8]. For thiols temperatures high as 20°C are more favour for their modulation, whereas low temperatures around 13°C show significant less modulation of the 3MH [63].

3.2. Nitrogen source (yeast assimilable nitrogen (YAN)) effect on aroma formation

Yeast assimilable nitrogen (YAN) concentration in grape must is a vital parameter not only completion of the fermentation but also for the production of volatile and nonvolatile metabolites [64, 65]. The depletion of YAN in grape juice during the early stages of fermentation is also triggering the entry to the stationary phase of yeast growth [35]. YAN, source in grape must is categorised into two types, the organic and the non-organic. The organic fraction, often referred to free amino (or amino acid) nitrogen FAN, is the total amount of the amino acids and some small peptides that can be utilised by the yeast. Ammonium nitrogen is the inorganic fraction. An initial concentration of 140 mg/L of YAN in the grape juice is considered to be the lowest threshold for the completion of an industrial fermentation, with low fermentation temperatures and low suspended solids [35]. Measurement of the initial YAN and supplementation of ammonium salts or mixtures of amino acids, to reach the lowest threshold of concentration, is a common practice in most of the wineries, as a prevention measurement to sluggish or incomplete fermentations. Supplementation of nitrogen during the early stages or even through the fermentation course not only results in high fermentation kinetics and yeast growth but also to the formation of various volatile and non-volatile compounds [66, 67]. Timing of the nutrition supplementation is also important since this is influencing the type of nitrogen intake by the yeast cell [35, 65]. Specifically ammonium ion has an inhibitory role in the uptake of amino acids, since in high concentration at the early stages of growth, the general amino acid permease (GAP) is not synthesised [15, 16]. This results in low uptake of amino acid during later stages of fermentation. Another parameter that has an inhibitory role in the amino acid uptake by the yeast is the CO_2. High pressure of CO_2 was observed to reduce the rate by which the amino acids are absorbed. Wines with high concentration levels more than 300 mg/L have showed high esters concentration and low concentrations of acids and higher alcohols [35]. The basic information regarding the initial concentration and ratio between organic and non-organic nitrogen can be obtained by a rather easy enzymatically or chemical method. Nature of the YAN, organic or not, plays also an important role in the outcome of the volatile profile [67]. Addition of amino acids in order to increase the YAN in low concentration grape juice, under the current regulation is forbidden. Use of amino acid enriched dry yeast preparation can provide the mean to serve this purpose; also these types of preparations are high in small peptides. Ratio between the two nitrogen sources is a good tool for the winemaker, to modify the aroma profile composition of the produced wine. It has been proven that the type of nitrogen supplementation resulted in quantitative differences for most of yeast metabolites related compounds, suggesting the importance of the supplementation decision-making process [35]. The concentration of acetates and medium-chain fatty acid esters, contributing to the fruity aroma, is favoured by the higher concentration of amino acids rather than ammonium concentrations. Also higher amino acid concentration is leading to higher concentration of fusel alcohols. High concentrations of ammonium as the sole nitrogen supplement, results in the increase of ethyl acetate and acetic acid [35, 64]. Other recent studies show that there is a close relation between the initial concentration and also most importantly profile of various amino acids for the production of certain aroma profile. Also the same study gives importance values to specific amino acids. Namely in the case of *S. cerevisiae*, they are leucine, isoleusine, valine, histidine, glutamine and proline under certain

conditions. [35]. Whereas other researchers' show that, for the formation of volatile compounds: threonine, phenylalanine and aspartic acid are amino acids with the most important value. For thiols, high addition of assimilable nitrogen in the early stage of fermentation, in the form of ammonium (di-ammonium phosphate) seems to reduce the 3MH production [68]. In [56], researchers documented that the highest concentration of terpenes is obtained under conditions that stimulate glycolytic flux. Microaerobic and high assimilable nitrogen conditions, favour terpene accumulation.

3.3. Suspended solids and contribution to wine aroma

During the process of vinification and especially during the first stages of destemming and pressing it is inevitable the presence of grape solids in the must. These solids of various, origin, nature and size are generally referred to as 'sludge' [18]. The measurements by the winemakers assess the presence of these solids are the turbidity units (nephelometric turbidity units, NTU) and a wt% on suspended solids (total wet suspended solids, TWSS or dry TDSS% (w/w) [18]. The ease to measure NTU makes this measurement, the most widely used and accepted method of reference in wineries. A limitation of NTU measurement is that is not in a direct relation with the suspended solids quantity since this is a nephelometric measurement and is being influenced by the size and shape of the particles and refractive index of the medium [18]. Also NTU does not give us information regarding the composition of the suspended solids. Must suspended solids can influence white wines aroma profile in many different ways, directly and indirectly. Suspended solids are consider a good nutrient source, specially for amino acids, the role of which is consider to be the most crucial of all must substrates for the formation of volatile compounds [18–21, 69]. Another direct role of suspended solids is the high content of oxidative enzymes, which is enhanced by various grape moulds contaminations. Also some evidence suggests that grape tissues contain esterase, a limitating factor for the accumulation and final concentration of esters at the final product. Presence of SS on fermentation apart from the direct role on chemical composition, have also an important indirect role, that of the nucleation of the CO_2 [18] and the further release it to atmosphere. High accumulation of CO_2 produces higher concentrations of acetic acid by limiting the long chain fatty acid synthesis. Also over oversaturation of CO_2, is affecting the transport and utilisation by the yeast of amino acids [18].

3.4. Inoculation rate and contribution to wine aroma

Yeast starting cultures are extensively used by many wineries as a mean to control the course of fermentation avoiding slow or sluggish fermentations [13]. Now available on market are a large number of dry yeast cultures ready to use. Primarily the need to use is the easy completion of fermentation, without any technological folds like reduction smell SO_2 and high concentration of volatile acidity. The size though of the inoculation it seems to play a catalytic role in the overall behaviour and physiology of the yeast during fermentation [11–13]. In some cases [12], it was observed that the size of inoculation enhanced stress protectants like glycerol and proline production in high inoculation rate. Also in the same study, an observation of the reduction of citric acid cycle intermediate metabolites was made. On another study dealing with three inoculation concentrations 1×10^4, 1×10^5 and 1×10^6 [11] were studied. It was

clear that the most favourable results, for the increase concentration desirable volatiles, like esters, and the simultaneous decrease of high concentrations of unfavourable volatiles, like higher alcohols was observed at inoculation rate of 10^5. It is obvious that through this process, of inoculation, the outcome of the fermentation can be altered but since there is not a lot of research done to this direction, is something that need to be investigated further.

4. Yeast autolysis

4.1. Yeast autolysis and contribution to wine flavour

At the final stages of winemaking, the settlement of yeast cells at the bottom of fermentation tanks is inevitable, since there is not any CO_2 production. From that stage on an autolysis of dead yeast cells is observed. During this period, the hydrolysis of cell wall is taking place, releasing various compounds that up to that moment were either part of yeast cell wall or were capture inside the cell cytoplasm. Understanding the nature of the cell wall is vital in this stage. Yeast cell wall is compromising around 15–25% of total dry cell mass [70, 71] depending on the growth conditions of yeast. Yeast cell wall consists of polysaccharides, inner layer, mannoproteins and outer layer [70]. Mannans and glucans consist of about the 94–98% of the total structural cell wall mass with a small fraction of chitin. Mannoproteins play an important role in stabilising various fractions of wine, tartaric salts and also proteins [72]. Moreover, for the red wines, this fraction can make wine to feel less astringent due to mannoproteins/tannin condensation (46, 48) and also increase the colour stability [72]. Apart from these, the autolysis can also influence the aroma character of the wine [73, 74]. First, by the absorption of volatiles like 4-ethylphenol and 4-ethylguaiacol, which they have a negative contribution to the wine aroma [74]. OAC compound profile also is affected by the yeast autolysis. The overall reduction in ester concentration is observed due to release of esterase [75]. But in some case, volatiles like diethyl succinate, vitispirane and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) levels are increased over the period of maturation of sparkling wine with the lees [76]. Also the reduction of oak character is observed due to the absorption of this group of volatiles by the yeast [75], in some case is considered to be positive and negative in some cases.

5. Future perspectives

Deep understanding of the volatiles chemistry in the matrix of wine, by the winemaker, is one crucial step understanding of the character of wine. With the current legislation, in most of the wine producing countries, allowing major taste alterations, like acidification, deacidification and fining, wine is subject to winemaker's decision regarding the final mouth feel. Never the less any additions of adjuncts, to alternate any of the characteristics of wine aroma are forbidden. The single most important period that the winemaker has a chance to manipulate or redirect the wine flavour during the period of fermentation. During this relatively short period to the wines life, the decision-making is critical. The decisions for the fermentation

temperature, addition of FAN and period of autolysis are tools for this purpose. The next step for this is monitoring the timing for the various procedures with the correlation across the various parameters. It is widely known and accepted that the quality of the wine is defined by the quality of the grapes. Although the new omics techniques employed in commercial yeast can give a lot of info not only regarding the physiology but most importantly to predict the direction of the outcome of fermentation, in terms of aroma profile, this has not yet put in industrial application. When these technologies become more available and affordable they will provide the winemaker with additional tools in order to improve the quality of the wine by addition of nutrients, adjustment of temperature, selection of commercial yeast strains in order to express at its best the character of the grapes that is handled.

In addition to the above, it is of current interest, research and development the role and use of other non-*Saccharomyces* yeasts from the native microbiota of grapes contributing to the complexity of wine aroma [59, 60, 77–79] and the geographical fingerprinting and indication of origin [57, 80, 81]. With these latest developments, it is understandable that it is impossible to proceed without good understanding of the yeasts physiological traits of many more genus, species and strains along the environment's role on them. For these reasons, high-throughput tools and instruments of molecular biology and biotechnology as well as of analytical chemistry are absolutely required to unravel the yeasts roles in aroma and flavour. These tools and instruments are in an exponential growth technologically and in downward trend their prices which are currently available in many laboratories around the globe and soon will be available for industrial application at large winery level.

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