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Formation of Aromatic and Flavor Compounds in Wine: A Perspective of Positive and Negative Contributions of Non-*Saccharomyces* Yeasts

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Abstract

Wine is a complex matrix that involves compounds of different chemical nature, with volatile compounds being primarily responsible for the aromatic quality of the wine. The formation of these volatile compounds is mainly due to yeasts' metabolism during alcoholic fermentation. Several studies in the microbiology field have reported that *Saccharomyces cerevisiae* is responsible for alcoholic fermentation, influencing the sensory quality of the wine and affecting the metabolic activity of other genera and species of yeasts, called non-*Saccharomyces*, which would positively affect sensory quality. Non-*Saccharomyces* yeasts, considered until recently as undesirable or spoilage yeasts, can improve the chemical composition and aroma profile of the wine. The activity of these yeasts is considered essential for the final wine aroma profile. Thus, the metabolism of these microorganisms could be a decisive factor that strongly influences the aroma of the wine, impacting on its quality. However, there are few studies that explain the impact of non-*Saccharomyces* yeasts on the final wine aroma profile. This chapter summarizes relevant aspects and pathways involved in the synthesis of aromatic compounds by non-*Saccharomyces* yeasts as well as studies at the genetic and transcriptional level associated with their formation.

Keywords: wine, non-*Saccharomyces* yeasts, fermentation, aroma, flavor

1. Introduction

The study of the yeasts involved in the wine fermentation process has shown that the main yeasts genera are *Saccharomyces*, *Candida*, *Debaryomyces*, *Hanseniaspora*, *Kloeckera*, *Pichia* and *Torulaspora* [1]. Despite this large number of genera involved in this process, it has been identified that the species responsible for alcoholic fermentation is *Saccharomyces cerevisiae*.

Starter cultures of *S. cerevisiae* are currently used by winemakers in order to homogenize the microbiota and to prevent unwanted yeast species from prevailing in the fermentation process. These cultures quickly position themselves against the rest of the yeasts, thus ensuring the quality of the final product without being conditioned by the other microorganism species present in the fermentation.

Due to the importance of the aromatic products obtained during the fermentation process, numerous works have been carried out correlating the strain of *S. cerevisiae* used versus the aroma of the wine obtained [2]. Thereby, several studies carried out in the field of wine microbiology have reported that not only *S. cerevisiae* has an effect on the sensory quality of the wine, but also the metabolic activity of other genera and species of yeast, called non-*Saccharomyces*, would positively affect sensory quality.

In this way, the sequential action of these different genera and yeast species contributes to the wine aroma and flavor, determining the final sensory quality. The wine aroma and flavor are mainly obtained by many volatile compounds formed during the alcoholic fermentation, including alcohols, esters, organic acids, phenols, thiols, monoterpenes and norisoprenoids.

In this context, *Candida stellata* and *Kloeckera apiculata* stand out for their high glycerol production. This compound provides sweetness and fullness in wines, but the perception of these sensations depends on the concentration and on the wine [3].

Candida colliculosa stands out for its production of acetaldehyde and n-propanol, which can have a positive influence on the quality of the wine. Likewise, other non-*Saccharomyces* species possess β -glucosidase activity, an enzyme that can hydrolyze aromatic precursors [2, 4]. In this way, the initial activity of these yeasts in the must is considered essential for the final wine aroma profile, because they are responsible for different reactions in the development of a wide range of volatile and nonvolatile products. As mentioned before, partially, it has been reported that the use of non-*Saccharomyces* yeasts in wine fermentation improves several parameters associated with the final wine quality, such as the increase in glycerol content [5], aromatic complexity [6], acidity [7] and anthocyanin content [8].

However, despite the aromatic potential of non-*Saccharomyces* yeasts, most of them have a low fermentative capacity, due to their low tolerance to alcohol, being unable to finish the fermentation. These characteristics have limited their use in the industry, despite their potential.

Currently, one of the strategies is the use of mixed cultures of non-*Saccharomyces* species with *S. cerevisiae* strains. This represents a useful tool that allows taking advantage of the sensory qualities of non-*Saccharomyces* species and the fermentative fitness of *S. cerevisiae*, favoring the sensory complexity and, therefore, the quality of the wine obtained [9]. Studies of mixed cultures of *Candida cantarelli* and *S. cerevisiae* reported that the use of sequential inoculation of these yeasts contributes to the improvement of the sensory characteristics of Syrah variety wine [10]. Likewise, Jolly et al. [4] observed that the aromatic profile of Chenin Blanc wines was improved with mixtures of *Candida pulcherrima* and *S. cerevisiae*. García et al. [11] reported similar observations for Chardonnay wines. Regarding fermentation of musts with a high concentration of sugar, it has been reported that the use of *T. delbrueckii* and *S. cerevisiae* reduced the volatile acidity and improved the analytical profile of the wine [12]. Also, the combined use of *Debaryomyces vanriji* and *S. cerevisiae* increased the concentration of geraniol [13]. An increase in varietal thiols was observed in cofermentation with *Pichia kluyveri* and *S. cerevisiae* [14]. Clemente-Jimenez et al. [15], using sequential inoculum of *Pichia fermentans* and *S. cerevisiae*, observed an increase in the concentration of specific aromatic components.

These results show that there is a huge potential for the application of non-*Saccharomyces* yeasts in oenology and strong evidence that their use contributes to the production of specific volatile compounds improving the aromatic composition of wines (**Table 1**).

Species	Metabolites		References
	Increase	Decrease	
<i>Saccharomyces cerevisiae</i>	Acetaldehyde Ethyl esters (caprylate)		[16, 17]
<i>Torulaspora delbrueckii</i>	Fruity esters Ethyl propanoate Ethyl isobutanoate Ethyl dihydrocinnamate Thiols Terpenes Glycerol 3-methylthio-1-propanol 4-MSP	Acetic acid Acetaldehyde Ethanol Higher alcohols	[5, 6, 18–30]
<i>Kluyveromyces spp</i>	Lactic acid Esters Monoterpenic alcohols 2-phenylethanol Carboxylic acids Ketones Furans Isoamyl acetate	Acetic acid	[31, 32]
<i>K. marxianus</i>	Polygalacturonases 2-phenylethanol Phenethyl acetate Ethyl acetate		[33–35]
<i>K. lactis</i>	Monoterpenoids		[36–40]
<i>Hanseniaspora spp</i>	Acetic acid Acetate ester Ethyl acetate Sulfur compounds Hydrogen sulfide		[16, 41]
<i>H. uvarum</i>	Acetic acid		[42]
<i>H. guilliermondii</i>	Acetate ester		[43]
<i>H. vineae</i>	Acetate and ethyl ester 2-phenylethyl acetate β-damascenone Isoamyl acetate Phenylacetaldehyde	2-phenylethanol	[44–48]
<i>Metschnikowia pulcherrima</i>	Free terpenes Linalool Geraniol Nerol Citronerol Alpha-terpineol Biogenic amines (histamine, tyramine and putrescine) Acetate esters β-damascenone Higher alcohols (isobutanol and phenylethanol)	C6 alcohols	[48–54]
<i>Brettanomyces bruxellensis</i>	Volatile phenols (4-ethylphenol) 2-acetyl-3,4,5,6-tetrahydropyridine 2-acetyl-1,2,5,6-tetrahydropyridine 2-ethyl-3,4,5,6-tetrahydropyridine Isoamyl alcohol Isoamyl acetate Esters		[55–60]

Species	Metabolites		References
	Increase	Decrease	
<i>Schizosaccharomyces</i> spp	H2S Acetaldehyde Pyruvic acid 2, 3-butanediol Acetoin Acetic acid	Esters Higher alcohols Gluconic acid	[61–70]

Table 1.
Metabolites produced in wine by non-Saccharomyces yeasts in mixed fermentations compared to fermentations with S. cerevisiae.

2. Wine aromas produced by non-Saccharomyces yeasts

The formation of aromatic compounds has been extensively studied in *S. cerevisiae*. In this regard, higher alcohols are synthesized from amino acids by transamination and decarboxylation reactions (**Figure 1**). Permeases of amino acids participate in these reactions, which are encoded by the *GAP1*, *BAP2* and *MEP2* genes. Subsequently, the transamination reactions are carried out by enzymes encoded by the *BAT1* and *BAT2* genes, which code for branched-chain amino acid transaminases, and the *ARO8* and *ARO9* genes that code for aromatic amino acid aminotransferases, which catalyze the transfer of amines between amino acids and their respective α -keto acid. Subsequently, the decarboxylation reactions of the α -keto acid occur to form the respective aldehydes, where the *PDC1*, *PDC5*, *PDC6*, *THI3* and *ARO10* genes are responsible for coding for enzymes with decarboxylase activity and, finally, dehydrogenases act, which reduce aldehydes to alcohols, a reaction that is carried out by alcohol dehydrogenases, encoded by the *ADH1–7* and *SFA1* genes, and aryl alcohol dehydrogenases, encoded by *AAD* genes [71–73].

Other important compounds are acetate esters, and their synthesis occurs by condensation between higher alcohols and acetyl-CoA (**Figure 2**). This reaction is carried out by acetyltransferases, encoded by the *ATF1* and *ATF2* genes. The ethyl esters are produced by condensation between ethanol and acyl-CoA, a reaction mediated by acyltransferases encoded by the genes *EHT1*, *EEB1* and *YMR210W*, encoding for a monoacylglycerol lipase [74].

Likewise, it has been reported that *S. cerevisiae* participates in the primary release of aromas through the activity of glucosidase enzymes [76].

2.1 *Torulaspora delbrueckii*

Among the non-Saccharomyces yeasts, *T. delbrueckii* has gained interest in the vitiviniculture industry because it modifies the aromatic properties of final wines in a very positive way, producing higher levels of fruity esters, thiols and terpenes and lower amounts of higher alcohols, thus respecting the initial character of the grape [6, 18, 19]. Also, *T. delbrueckii* typically produces low concentrations of acetic acid [12], one of the main quality parameters in wine production. It has also been reported that *T. delbrueckii* produces wines with higher levels of glycerol [5] and, consequently, with lower concentrations of ethanol [20]. This is currently a relevant feature because as a consequence of climate change, an increase in sugar concentration in the must has been observed, resulting in wines with higher alcohol content.

During alcoholic fermentation, the ethanol production is usually higher than 12% (v/v), so the associated microorganisms must have resistance mechanisms for this compound. In practice, the phenotype of ethanol resistance among wine yeasts

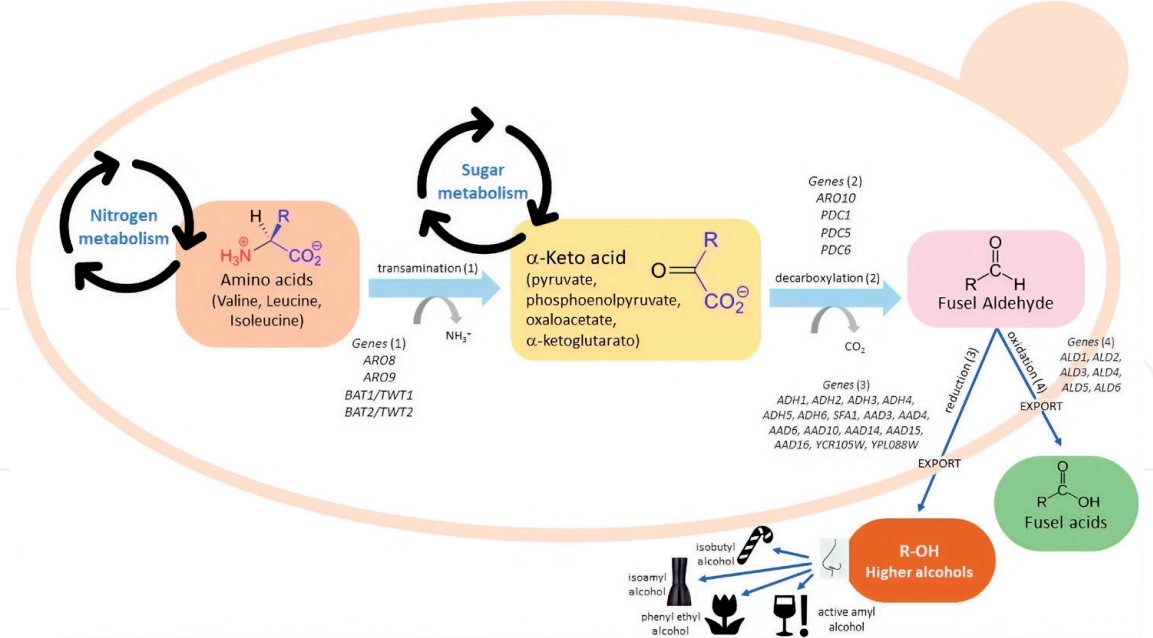


Figure 1.
Ehrlich pathway for higher alcohol production (adapted from [71]).

is heterogeneous, *S. cerevisiae* being the one with the highest level of resistance and the one in charge of leading the alcoholic fermentation. However, non-*Saccharomyces* yeast species play an important role during the early stages of spontaneous alcoholic fermentation, when the ethanol concentration is not very high [77].

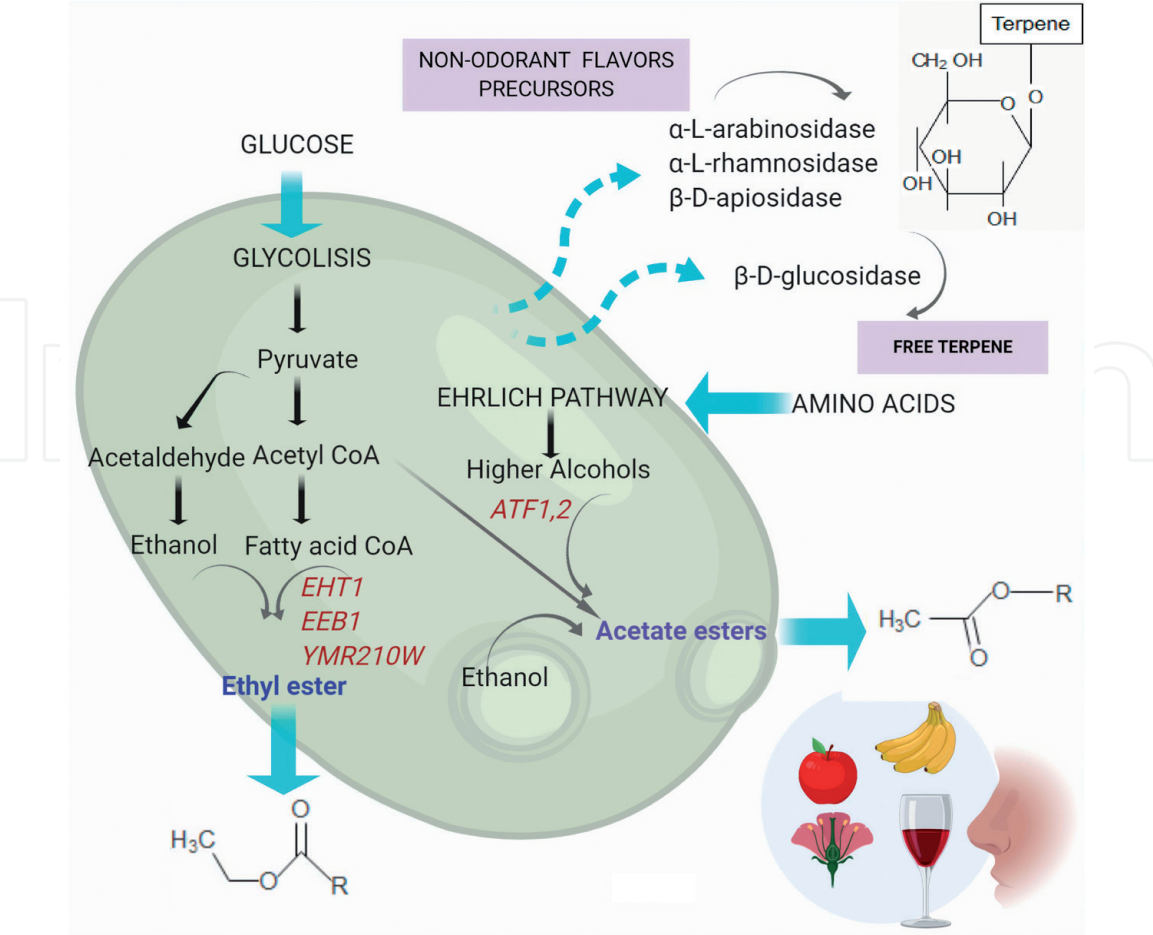


Figure 2.
Acetate ester and ethyl ester biosynthesis (adapted from [75]).

Therefore, currently, the strategy of mixed and/or sequential fermentations is used, which combines non-*Saccharomyces* yeasts with a yeast with a higher fermentative profile such as *S. cerevisiae*, which in most of the cases is necessary to properly end the industrial process of alcoholic fermentation.

T. delbrueckii has been described as capable of fermenting and tolerating up to an ethanol concentration slightly higher than 9% (v/v) [21]. On the other hand, Bely et al. [12] have reported that this value is lower, reaching only 7.4% (v/v). Nevertheless, Belda et al. [5, 6], through studies of population kinetics in sequential fermentation, observed that *T. delbrueckii* suffered a significant decrease in the cellular viability when ethanol levels exceed 8% (v/v). This suggests that the ethanol resistance of *T. delbrueckii* is limited and much lower than that of *S. cerevisiae*, which complicates its use in industrial fermentations. Nevertheless, to improve the fermentation rate of the selected nonconventional yeasts, sequential cultures are used, but this is to the detriment of the diversity of aromas that could be present in the final product. Given this context, ethanol resistance is an important factor in the selection of industrial non-*Saccharomyces* strains and particularly of *T. delbrueckii*.

Non-*Saccharomyces* yeast species can produce the aromatic volatiles that are known to be important for industrial beer and wine fermentations and that are produced by *Saccharomyces* species [78]. For the case of *T. delbrueckii*, several studies have indicated how beneficial its, from the aromatic point of view, incorporation into fermentations is [79–81]. Belda et al. [6], evaluating a sequential fermentation using Verdejo variety must, observed a higher aroma quality, intensity and fruity character. Chromatographic analysis indicated that this effect was due to an increase in the levels of the main ones, mainly 4-methyl-4-sulfanyl-pentan-2-one (4-MSP), which is represented in this grape variety. Likewise, Renault et al. [22] reported that mixed inoculations of *T. delbrueckii* and *S. cerevisiae* allowed the increase of some esters specifically produced by *T. delbrueckii*, which correlated with the maximum population reached by it in mixed cultures. Among the reported compounds were ethyl propanoate, ethyl isobutanoate and ethyl dihydrocinnamate, which are considered activity markers for this yeast.

The signaling pathways involved in the formation of aroma and flavor compounds, such as the Ehrlich pathway, or the specific enzymes responsible for the synthesis of ester, are also present in nonconventional yeast. This route has been studied extensively in *S. cerevisiae* [71, 82, 83]. This pathway consists of a step of transamination of amino acids to α -keto acids, followed by decarboxylation to “fusel aldehydes.” These fusel aldehydes can be reduced or oxidized in fusel alcohols or fusel acids, respectively [71, 84]. Subsequently, aromatic esters can be formed from alcohols and fusel acids [23], and these compounds are responsible for the characteristic aroma and flavor of the final fermented product [72]. In addition, these aromatic esters have a low detection threshold, which is why minimum amounts of these compounds are required for the perception of the human olfactory senses [23].

It has been reported that the concentration of assimilable nitrogen has a significant effect on the production of fermentation aromas [85, 86]. A higher concentration of higher alcohols at the end of fermentation has been observed in media with low nitrogen content [24–26]. Likewise, there is a directly proportional relationship between the concentration of nitrogen and the synthesis of the ethyl esters, in which the initial content of nitrogen is associated with an increase in the production of esters [27, 28]. In this sense, Bloem et al. [87] observed that the nitrogen composition of the medium could influence the redox balance in the yeast cells during alcoholic fermentation and that variations in this balance could change the final concentrations of certain volatile compounds. Changes in the levels of these compounds were closely related to the effects of redox status on the availability of

acetyl-CoA, an intermediate of central carbon metabolism and precursor of α -keto acids. Similar results were reported by Rollero et al. [88] who observed that a small change in the acetyl-CoA pool would affect the bioconversion of acetate esters from higher alcohols. These results suggest that it is possible to increase the aromatic potential of *T. delbrueckii* by modulating the availability of nitrogen in the medium, which would influence the redox balance of the cells directly affecting the final concentrations of certain volatile compounds.

Through next-generation sequencing, Tondini et al. [89] characterized the transcriptome of *T. delbrueckii* COFT1 observing differences in glucose fermentation pathways and the formation of aromatic and flavoring compounds, such as glycerol, esters and acetic acid with respect to *S. cerevisiae*. These differences are partly explained by the absence of paralogous genes in glycolysis and glycerol biosynthesis in *T. delbrueckii*. It has been reported that *T. delbrueckii* produces less acetic acid [29], and this phenomenon depends on increased expression of genes related to alcoholic fermentation, while acetate ester levels were influenced by the absence of esterases, *ATF1*–*2*. Likewise, a lower production of ethyl esters was observed in *T. delbrueckii* COFT1, which suggests a negative regulation in the fatty acid pathway biosynthesis.

2.2 *Kluyveromyces* spp.

Kluyveromyces species do not usually intervene in spontaneous fermentation processes because they have a low fermentation capacity and slow multiplication [31]. However, they are capable of producing considerable amounts of lactic acid (1.5–1.8 g/L) and low amounts of acetic acid. It has been reported that *Kluyveromyces* species produce aromatic compounds such as esters, monoterpenic alcohols, carboxylic acids, ketones, furans and isoamyl acetate in liquid phase fermentation. Of all these compounds, the production of 2-phenylethanol (2-PE) stands out [32], with the aroma of rose petals, which is commercially important, since it gives characteristics that positively influence wine quality, among others [90]. In particular, the influence of the carbon source [91, 92], the aeration rate [92], the composition of the medium [93] and growing conditions [94] on the production of aromas in *K. marxianus* has been studied.

K. marxianus produces polygalacturonases, enzymes that added in the fermentation of musts favor the release of aromatic compounds, resulting in citrus, balsamic and floral wines [33]. Other studies have demonstrated the fermentation capacity of *K. marxianus* in pure culture for the production of tequila; however, in mixed cultures with *S. cerevisiae*, the activity of *K. marxianus* is negatively affected [95].

Another group of important aromatic compounds is monoterpenoids. The common precursor of these compounds is geranyl pyrophosphate (GPP). Although plants, such as *Vitis vinifera* and *Humulus lupulus*, produce monoterpenoids [36], it has been reported that yeasts can also produce them [37], highlighting *K. lactis* [38–40].

Marcišauskas et al. [34], using the strain of *K. marxianus* iSM996, constructed the first genome-scale metabolic model for this yeast. This model contains several unique biosynthetic pathways for aromatic compounds such as 2-PE, phenethyl acetate and ethyl acetate. The *K. marxianus* iSM996 model is a solid tool to evaluate the metabolic characteristics of *K. marxianus*, allowing the integration of experimental data and strain design based on the model.

Ivanov et al. [35] studied the production potential of 2-PE by the strain of *K. marxianus* 35. The results revealed that the enzymatic activity of aminotransferase, pyruvate decarboxylase and alcohol dehydrogenase, key enzymes of the Ehrlich pathway, was almost twice as large compared to *S. cerevisiae*. In addition, the

residual concentration of 2-PE was twice lower in *K. marxianus* 35 and the efficiency was found to be 73% for this strain. Additionally, the sequence variability in the genes encoding the key enzymes of the Ehrlich pathway suggests that in addition to the physiological advantages *Kluyveromyces* have probably undergone substantial evolutionary genetic alterations that result in higher enzymatic activities and a better transformation potential of 2-PE.

2.3 *Hanseniaspora*

Species of the genus *Hanseniaspora* are ubiquitous in the winemaking environment, and some of them have been proposed as wine yeast starters [96].

Fermentations of mixed cultures by wild yeasts, such as *H. guilliermondii*, together with *S. cerevisiae* have shown higher concentrations of acetate ester compared to fermentations with *S. cerevisiae* alone, without significantly affecting acetaldehyde, acetic acid, glycerol and higher total alcohols [43]. However, Lleixà et al. [44] reported that the use of the *H. vineae* species as an initiator is capable of granting aromatic complexity in wines, producing key aromatic compounds. However, the sensory evaluation of the wines produced by this apiculate yeast is still limited and the results have not been consistent. In this regard, Medina et al. [45] reported that fermentation using *H. vineae* produced up to 10 times higher levels of 2-phenylethyl acetate in wine, compared to conventional and spontaneous fermentations. However, the opposite was observed for the concentration of 2-PE, which was significantly lower. Similar results were reported by Viana et al. [46, 47], regarding the high production of 2-phenylethyl acetate by *H. vineae*.

It should be noted that the aromatic contribution of 2-PE is controversial. Fuente-Blanco [97] reported that the contribution of 2-PE in the aroma of red wine was insignificant, in addition to depending on the aromatic context.

On the other hand, Viana et al. [16] reported that *Hanseniaspora* spp. produce high levels of ethyl acetate. In this regard, it is important to highlight that ethyl acetate at low levels, below 80 mg/L, confers aromatic complexity on the wine, giving it a “fruity” aroma. However, over 150 mg/L is responsible for the typical altered sensory properties of acescence [98].

The acetic acid concentration in wines is also important, becoming a defect near its flavor threshold of 0.7–1.1 g/L. Some *H. uvarum* species have been reported to produce acetic acid levels of up to more than 3.4 g/L [42].

Other compounds have been associated with the metabolism of *H. vineae* such as β -damascenone, isoamyl acetate and phenylacetaldehyde, which have been identified in ice wine fermentations [48].

Seixas et al. [99] reported the reconstruction of the metabolic network for *H. guilliermondii* UTAD222, noting that this strain of yeast contains four genes that code for β -glucosidases, as well as the genes necessary for the synthesis of acetaldehyde, ethyl esters and higher alcohols. Surprisingly, no *S. cerevisiae* acetyl transferase-like proteins, involved in the synthesis of acetate esters, were found in the ORFeome of *H. guilliermondii* UTAD222. This is contradictory because it has been described that the synthesis of these compounds is high in this species [43]. Likewise, no sequences associated with aryl alcohol dehydrogenases were found, enzymes necessary for the synthesis of higher alcohols from aldehydes, which could contribute to the lower reported capacity of this species to produce these compounds, especially in comparison with *S. cerevisiae*.

Giorello et al. [100] recently reported genome sequencing, assembly and phylogenetic analysis of two strains of *H. vineae*. When these genomes were compared with 14 genomes of *S. cerevisiae*, specific flavor gene duplications and absences

were identified in the *H. vineae* genome. In this regard, the increase observed in the formation of 2-phenylethyl acetate and phenylpropanoids, such as 2-phenylethyl and benzyl alcohol, could be explained by duplications of *ARO8*, *ARO9* and *ARO10* genes. Similarly, the high level of acetate esters produced by *H. vineae* compared to that of *S. cerevisiae* is related to the identification of six proteins with domains of alcohol acetyltransferase (AATase). The opposite occurs with the reduced production of higher branched chain alcohols, fatty acids and ethyl esters, which responds to the absence of branched chain amino acid transaminases (*BAT2*) and acyl coenzyme A (acyl-CoA)/ethanol O-acyltransferases (*EEB1*).

2.4 *Metschnikowia*

Metschnikowia pulcherrima is one of the non-*Saccharomyces* yeast species with the greatest capacity to express extracellular hydrolytic enzymes. In *M. pulcherrima*, the presence of enzymes with pectinase, protease, glucanase, lichenase, β -glucosidase, cellulase, xylanase, amylase, sulfite reductase, lipase and β -lyase activity [49, 101–103] has been described. Also, its high proteolytic activity makes it a candidate to be used in fermentations with *S. cerevisiae*, releasing amino acids and increasing the available nitrogen sources for the growth of *S. cerevisiae* [104, 105]. It also stands out for its glucosidase-dependent strain activity [106, 107], which increases in aerobic conditions [50], promoting the release of varietal aromas by hydrolyzing bound monoterpenes. The expression of β -D-glucosidase favors the release of free terpenes and this activity has been evaluated using the 4-methylumbelliferyl- β -D-glucoside (MUG) and p-nitrophenyl- β -D-glucoside (pNPG) substrates [108].

Terpenes are relevant in the varietal character of various white grape varieties, being the main descriptors of varieties such as Muscat, Riesling or Alvarinho [51]. Their presence and relevance in certain red grape varieties are also specific. However, the composition of free terpenes in the must is scarce, with a large amount of glycosylated terpenes [52]. These can be released by enzymatic hydrolysis by glycosidase enzymes [53, 109]. Within this group, linalool, geraniol, nerol, citronellol and α -terpineol stand out [51, 53].

The enzymatic hydrolysis of glycosides is mainly carried out by several enzymes that act sequentially, according to two steps: first, α -L-rhamnosidase, α -L-arabinosidase or β -D-apiosidase make the cleavage from terminal sugar and rhamnose, arabinose or apiose and the corresponding β -D-glycosides are released. Subsequently, the release of terpene occurs after the action of a β -D-glucosidase [110].

Likewise, mixed fermentations between *M. pulcherrima* and *S. cerevisiae* have identified higher levels of acetate esters and β -damascenone, and lower levels of C6 alcohols in ice wines of Vidal Blanc grape variety [48]. Similarly, a higher production of higher alcohols has been reported, with a greater amount of isobutanol and phenylethanol [54].

Another aspect to highlight for *M. pulcherrima* is that it has the ability to produce biogenic amines (histamine, tyramine and putrescine); however, this phenomenon would be strain dependent [49].

To date, only the genome of one *M. pulcherrima* strain has been reported [111], and genetic studies are scarce. Reid et al. [103] identified and characterized the gene that codes for an aspartic protease of *M. pulcherrima* IWBT Y1123, called MpAPr1. The results indicated that this protein presented homology with proteases of the yeast genera. Likewise, aspartic protease activity was confirmed by heterologous expression in *S. cerevisiae* YHUM272. This gene was found in 12 other strains of *M. pulcherrima*; however, analyzes revealed that the intensity of the enzyme activity was strain dependent and was not related to the gene sequence.

2.5 *Brettanomyces* spp.

The yeast *Brettanomyces bruxellensis* is one of the main contaminant yeasts in wines, with the ability to metabolize hydroxycinnamic acids, which are naturally present in grapes, into volatile phenols [55, 56]. It has been described that this yeast can grow in various stages of wine production, for example, after alcoholic fermentation, during malolactic fermentation, during maturation in barrels or in already bottled wine. This characteristic is due to its ability to tolerate high ethanol variables [57].

Volatile phenols represent a large family of aromatic compounds where vinyl and ethyl derivatives are involved with product deterioration [55, 58]. These volatile phenols, especially 4-ethylphenol, are responsible for odors that have been described as “animal,” “medicine,” “leather” and “stable,” which at concentrations above their perception threshold are detrimental to the aromatic profile of wines [55, 58].

The production of these compounds by *Brettanomyces* spp. is the result of the enzymatic transformation of hydroxycinnamic acids (3-methoxy-4-hydroxycinnamic acid (ferulic acid) and 4-hydroxycinnamic acid (*p*-coumaric acid)) by the action of two specific enzymes: cinnamate decarboxylase (CD) and vinylphenol reductase (VR) [112–115]. Also, *Brettanomyces* yeast species are capable of producing 2-acetyl-3,4,5,6-tetrahydropyridine, 2-acetyl-1,2,5,6-tetrahydropyridine and 2-ethyl-3,4,5,6-tetrahydropyridine. These compounds are responsible for “mousy taint” produced by microorganisms in the presence of lysine and ethanol [59].

It has been described that the ability of these yeasts to produce volatile phenols is variable [116, 117]. Factors such as the pH of the wine, the concentration of sugar and the moment in which this yeast is inoculated influence this capacity [118]. Along with this, it has been observed that the production of 4-ethylphenol in red wines is related to population growth, a phenomenon that would be strain dependent [119].

From the genetic point of view, there is a great intraspecific diversity of strains of *B. bruxellensis* [120–123], which translates into the different phenotypes of production of reported volatile phenols. The number of chromosomes in this species can vary between 4 and 9, with chromosome sizes in the range of 1 to 6 Mb, and total genome size between 20 and 30 Mb [124, 125]. Also, karyotypic studies suggest speciation due to genome rearrangements. However, available genetic studies are of a limited number of strains [121, 126–130]. In this regard, a transcriptomic analysis of the strain of *B. bruxellensis* LAMAP2480 exposed to *p*-coumaric acid indicates that this acid generates a stress condition, inducing the expression of the proton pump together with the output of toxic compounds, as well as the output of nitrogen compounds, reducing intracellular concentration and triggering the expression of nitrogen metabolism genes (**Figure 3**) [121].

Additionally, sequencing and genome analysis of the strain of *B. bruxellensis* AWRI1499 reported the presence of three homologous proteins with the isoamyl acetate hydrolysis enzymes of *S. cerevisiae* that are related to isoamyl alcohol concentrations and isoamyl acetate produced in fermentation. This strain was evaluated under fermentation conditions in model wine and produced higher levels of esters [60]. In this sense, it has been described that the formation of esters between *Brettanomyces* strains is variable.

The positive aromatic contribution of these yeasts has been studied mainly in beer. *Brettanomyces* spp. are able to esterify medium and long chain fatty acids in their respective esters, influencing the sensory profile of beers. Likewise, it has been reported that *Brettanomyces* has β -glucosidase activity, which would be responsible for breaking down the cellobiose present in the barrels, explaining its survival during the wine aging stage [131]. Crauwels et al. [120] reported that *B. bruxellensis* has

The contribution from the aromatic point of view of *S. pombe* has been recently reported where it has been observed that it stands out mainly for producing fewer amounts of higher alcohols in comparison to *S. cerevisiae*, which could be attributed as a strain-dependent characteristic [62, 63, 66, 68, 69].

Benito et al. [63] reported a lower production of isobutanol, 2-methyl-butanol, 3-methyl-butanol and 2-phenyl-ethanol in white wines by *S. pombe* in comparison to *S. cerevisiae*. Similar results have been reported by Mylona et al. [66] where, fermenting red must, they observed a decrease in 2-methyl-butanol, 3-methyl-butanol and isobutanol by *S. pombe* in comparison to *S. cerevisiae*. On the other hand, Chen et al. [69] observed that *S. pombe* possesses a special ability to produce more 2, 3-butanediol, which contributes to the fruity aroma described as banana to wines.

In the case of esters, a similar phenomenon occurs, observing that *Schizosaccharomyces* shows a tendency to produce lower concentrations of esters in comparison to *S. cerevisiae*. It has been reported to produce lower concentrations of isoamyl acetate and 2-phenyl-ethyl acetate in comparison to *S. cerevisiae* [62]. Likewise, lower production of total esters was reported by Del Fresno et al. [68] in comparison to *S. cerevisiae*.

Finally, *S. pombe* fermentations have been reported to show higher levels of acetoin in comparison to *S. cerevisiae* controls. Also, they are commonly associated with high levels of acetic acid. These levels might vary from strain to strain [68–70].

3. Conclusion

There are many physiological studies on the contribution of non-*Saccharomyces* yeasts to the aromatic profile of wines. However, reports at the genetic level that explain the differences observed in these yeasts with respect to *S. cerevisiae* are scarce.

Despite the little information available, it is possible to establish that the differences in aromatic potential observed in non-*Saccharomyces* yeasts are mainly due to modifications in the Ehrlich pathway and the biosynthesis of acetate esters and ethyl esters. These changes can be summarized as follows:

- a. Differences in the regulation of gene expression of these routes
- b. Absence of paralogous genes
- c. Gene duplications
- d. Modification of enzymatic activities

The identification of most of these biological mechanisms has been possible thanks to the use of massive sequencing technology (NGS).

Given the relevance of the contribution of non-*Saccharomyces* yeasts to the quality and typicity of wines and their impact on taste, more studies with genetic approaches that explain the metabolic diversity of these yeasts are required.

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Conflict of interest

The authors declare no conflict of interest.

Notes

Figure 2 was created with BioRender.

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