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Yeasts and wine colour

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ABSTRACT

Historically, yeasts from the genus *Saccharomyces* have been conventionally used in the production of wine and other fermented beverages. Traditionally, their main role has been the transformation of sugars into ethanol, however, research has shown that yeasts also influence wine aroma, texture, flavour and colour. In lieu of this, non-*Saccharomyces* yeasts, which have been considered as spoilage yeasts in the past, have been exploited as potential wine starters because they can improve the sensorial characteristics of wines. Because they are considered to be poor fermenters, mixed fermentations with *Saccharomyces* yeasts are applied either in a form of co-inoculation or sequential fermentation. Among wine characteristics, colour of red wines has special importance because it is the first wine characteristic perceived by the consumers. Red wine colour stems from anthocyanins, located in the grape skins that are extracted to grape must during maceration/fermentation. Various technological strategies in the winemaking process have already been employed to improve wine colour. One of them is yeast-mediated colour improvement employing a careful selection of yeast starters that can promote the synthesis of stable colour pigments pyranoanthocyanins from anthocyanins. The two most known groups of pyranoanthocyanins are vinylphenolic pyranoanthocyanins and vitisins. In comparison to anthocyanins they are less susceptible to pH, SO_2 bleaching and oxygen presence. Their concentration in the wines differs according to the yeast strain used and the type of fermentation applied. Furthermore, wine colour can also be influenced by the cell wall adsorption capability of yeasts. Numerous studies have shown the positive influence of a careful selection of non-*Saccharomyces* yeast in promoting stable pigments synthesis in the production of wine. In this review, we discuss how application of different yeast species – *Saccharomyces* and non-*Saccharomyces* can enhance wine colour through different fermentation strategies applied.

Introduction

In the production of fermented foods and beverages yeasts from the species *Saccharomyces cerevisiae* are conventionally used. They are used for the production of alcoholic beverages (as wine, beer, sake) and in production of bread (Steensels et al., 2014).
It is believed that wine has been produced as early as 6000 BC in Mesopotamia and the Caucasus. Later in history, the Romans spread winemaking practices in the Mediterranean regions, from where it also spread to the Balkan region and Germany. The wine has spread to the "New World" with European explorers in the 16th century. Although the production of wine has been known to humanity for thousands of years, the basic principles of wine production changed has not been changed much. In the last 150 years, the science behind winemaking has become clearer, resulting in improvements in winemaking and viticulture and oenological practices. The

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microbiological activity behind the winemaking becomes known in the 19th century when Louis Pasteur showed the role of yeasts for the commencement of wine fermentation; e. g. biotransformation of grape sugars into alcohol and carbon dioxide. This knowledge allowed to control the winemaking process and at the end of the 19th century concept of inoculating wine fermentations with pure yeast cultures was introduced, which resulted in better wine quality. Even though spontaneous fermentation without the use of starter cultures is still being used, in the large scale production of wine, inoculated wine fermentations are preferable because the fermentations are faster and more reliable, resulting in more consistent wine flavour and predictable quality (Pretorius, 2000). The notion that yeasts transform sugars to ethanol and carbon dioxide is rather simplistic because the advances in biotechnology, ecology, microbiology and analytical science showed that interactions between grape, wine and yeasts are very complex. The role yeasts play in the wine productions has been widened and goes beyond simplistic approach of transforming sugars into ethanol. Some of the interactions influence wine aroma, texture and flavour (Fleet, 2008; Suárez-Lepe & Morata, 2012; Ugliano & Henschke, 2009).

Yeasts associated with the winemaking

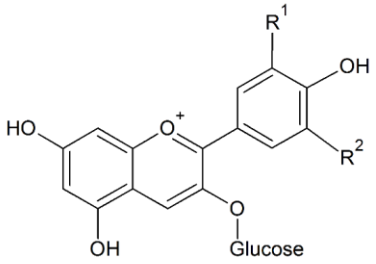
There are over 100 yeast genera that represent more than 700 species (Pretorius, 2000). The primary yeasts associated with winemaking are *S. cerevisiae*. *S. cerevisiae* is a single-cell fungus able to grow in high sugar and low pH environment. In contrast to other yeasts, especially non-*Saccharomyces*, they can survive in the presence of a high ethanol concentration. Therefore *S. cerevisiae* can ferment grape must, where concentrations of glucose and fructose are high (Swiegers & Pretorius, 2005).
Although *S. cerevisiae* is the most known species, more than 40 yeast species have already been identified from grape must (Jolly, Varela, & Pretorius, 2014; Marsit & Dequin, 2015). The most frequent non-*Saccharomyces* yeasts belong to the genera *Candida, Debaryomyces, Dekkera, Hanseniaspora, Kluyveromyces, Metschnikowia, Pichia, Schizosaccharomyces, Torulaspora,* and *Zygosaccharomyces* (Fleet, 2008; Marsit & Dequin, 2015; Pretorius, 2000). These yeasts originate from the winery environment and grape berries (Fleet, 2008).
Hanseniaspora, *Candida*, *Pichia*, and *Metschnikowia* species are known to initiate spontaneous alcoholic fermentation of grape juice, however, they are soon overtaken by *S. cerevisiae* which dominates until the final stages of the fermentation. Majority of non-*Saccharomyces* yeasts die off during fermentation which is associated with their slow growth and inhibition by the SO_2, low pH, high ethanol concentration and lack of oxygen (Ciani et al., 2006; Domizio et al., 2011; Jolly et al., 2014; Pretorius, 2000). This is also in accordance with their low fermentative power. Temperature is also an important factor that affects yeast growth (Ferreira et al., 2017; Salvadó et al., 2011). Furthermore, *S. cerevisiae* presence can also have a negative effect on the growth of non-*Saccharomyces* strains through quorum sensing and presence of killer toxins which can inhibit non-*Saccharomyces* yeast growth (Bagheri et al., 2017; Renault et al., 2013). Even though most of the non-*Saccharomyces* species die off during fermentation, there are some strains that are less sensitive to higher percentages of alcohol, the addition of SO_2 and capable of anaerobic and aerobic growth. In this case, non-*Saccharomyces* strains can compete with *S. cerevisiae* strains for nutrient growth and affect the aroma profile of final wine (Ciani et al., 2006; Domizio et al., 2011; Jolly et al., 2014; Pretorius, 2000).
Nowadays it is widely accepted that wine fermentation is a complex process, regardless of the type of fermentation performed (spontaneous *vs*. inoculated). Understanding how yeasts influence the key properties of the wine – aroma, flavour, and colour will provide the tool for selection of strains that could be potentially used as starters. For this purpose mixed culture fermentations of *Saccharomyces* and non-*Saccharomyces* strains are also being investigated either as co-inoculations or sequential inoculations (Clemente Jimenez et al. 2005; Comitini et al., 2011; Escott et al., 2018; Fleet, 2008).

Wine colour

Phenolic compounds are compounds that contribute to organoleptic characteristics of wine, especially colour, astringency, and bitterness. Phenolic compounds in wine can be divided into two groups; non-flavonoid and flavonoid phenolic compounds (He et al., 2012b). The flavonoids are the most abundant phenolic compounds in the grapes and wines. The flavonoid family is further divided into subgroups of flavones, flavanols, flavanones, and anthocyanins. Anthocyanins are glycosylated derivatives of anthocyanidins: delphinidin, petunidin, cyanidin, peonidin, and malvidin. Further diversity of wine/grape anthocyanins results from acylation of the glucose by hydroxycinnamic acids (caffeic acid, *p*-coumaric acid) or aliphatic acids such as acetic acid (He et al., 2010; Lorrain et al., 2013; Panche et al., 2016). Anthocyanins are natural pigments responsible

for the red, blue and purple colours of fruits, vegetables, flowers, and herbs. Their name derives from the Greek *anthos* which means flower and *kyanos* which means blue (Welch et al., 2008). The red colour of wines and grapes comes from anthocyanins. They are specific to red grape varieties, mainly located in grape skin, except in some grape varieties with coloured flesh. *Vitis vinifera* grapes contain mostly monomeric anthocyanins like -3-*O*-monoglucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin (Fernandes et al., 2017; He et al., 2010; Lorrain et al., 2013) (**Fig 1**). Their extraction yield during the winemaking process depends on the grape variety, maturity, seasonal conditions, production area, and fruit yield. The concentration of anthocyanins in wines depends also on the conditions of fermentation such as temperature during fermentation and maceration period. It is affected by the grape varietal differences, grape maturity and oenological practices. The ageing of wine also affects anthocyanin concentrations (He et al., 2012b; Mazza, 1995; Minnaar et al., 2018; Zou et al., 2002; Costa et al. 2014; Champ and Kundu-Champ, 2019).

Anthocyanins are not particularly stable biomolecules, because they are highly reactive and can be readily oxidized, especially in grape products and wines (He et al., 2010). In the highly acidic medium, they are usually present as very stable forms known as red flavylium cations. However, this is only in highly acid medium, usually below pH 2. In mildly acidic media, they are mostly present as quinonoidal base and in the hemiketal form. Therefore, at the wine pH, averaging at 3.5, the major anthocyanin (malvidin-3-*O*-glucoside) occurs mainly as the colourless hemiketal, meaning that the red flavylium cation, yellow chalcone, and blue quinoidal base are present as minor fractions (Cheynier, 2005; Es Safi & Cheynier, 2004). Aside from pH, the colour of anthocyanins is affected also by sulphur dioxide, which causes loss of its red colour (Bakker & Timberlake, 1997; Lee et al., 2004). The stability of anthocyanins is affected by different mechanisms such as self-association and co-pigmentation with other phenols present in wines (flavanols, flavonols, hydroxycinnamic acids) (He et al., 2010; Monagas et al., 2007). The co-pigmentation effect is a result of molecular associations between pigments and co-factors that are usually non-coloured. This causes the stabilization of anthocyanins and results in the colour enhancement. The colour exhibited by such complexes can be several times higher than the colour exhibited by anthocyanins. The actual enhancement of the colour depends on the pigment, pH, the cofactors and the ratio of a cofactor to pigment (Bimpilas et al., 2016; Boulton, 2001). This co-pigmentation phenomenon is more evident in young red wines (Fernandes et al., 2017).



Delphinidin-3-*O*-glucoside	R^1 = OH	R^2 = OH
Cyanidin-3-*O*-glucoside	R^1 = OH	R^2 = H
Petunidin-3-*O*-glucoside	R^1 = OCH^3	R^2 = OH
Peonidin-3-*O*-glucoside	R^1 = OCH^3	R^2 = OH
Malvidin-3-*O*-glucoside	R^1 = OCH^3	R^2 = OCH^3

Fig 1. Structures of the main anthocyanins present in grapes and wines

Caridi et al. (2017) tested six different *S. cerevisiae* yeast strains for the must fermentation of the *Gaglioppo* cultivar. Five of them were Calabrian strains (RC029-2CxRC039-3C(7)-1C, RC029-2CxRC039-3C(7)-3A, RC029-1DxRC039-3C(4)- 1A, RC029-1DxRC039-3C(4)-1C, RC026-3CxRC039-3C(9)-2B) and the sixth strain was commercial *S. cerevisiae* Zymaflore F15 strain obtained from Laffort Oenologie (France). The strains were chosen based on their different anthocyanin adsorption capacity. The resulting six wines differed in colour parameters, the instrumentally determined absorbance at different wavelengths (A420, A520, A620), colour intensity, Folin-Ciocalteu index, total polyphenols (A280) and total anthocyanins index. Some of the evaluated *S. cerevisiae* Calabrian strains contributed to higher colour intensity during bottle ageing in comparison to the wines produced with commercial strain Zymaflore F15. The results validate the assumption that yeast selection can play a role in the enhancement of the quality of red wines produced from low pigmented grapes (Caridi et al., 2017). Monagas et al. (2007) conducted a study on the influence of *S. cerevisiae* yeast strains on the anthocyanin, pyranoanthocyanins and non-anthocyanin phenolic compounds of red wines. They tested three native *S. cerevisiae* strains (1EV, 2EV, and 7EV) and commercial S. cerevisiae Na33/S. bayanus EC1118 (mixture 80/20) from Lallemand Inc. (Canada). The oenological properties of the tested strains were: ethanol tolerance, ≤16.0% v/v alcohol; volatile acidity production, <0.3 g/L expressed as acetic acid; glycerine production, >8.0 g/L; SO_2 tolerance, ≤200 mg/L; low production of

H_2S. The results showed that anthocyanins were the compounds most affected by the yeast strains independently of the grape variety (Monagas et al., 2007).

During wine maturation and ageing, anthocyanins react with other constituents present in wine, resulting in the formation of new anthocyanin-derived pigments which are polymeric and oligomeric pigments called pyranoanthocyanins. These compounds are responsible for the changing colour in wine during ageing (Alcalde-Eon et al. 2006; He et al., 2012a; Schwarz et al. 2003b) and have been shown to have higher colour intensity and increased stability as they are less susceptible to changes in pH, oxygen and have greater resistance to sulphur dioxide bleaching and temperature (De Freitas & Mateus, 2011; Marquez et al., 2013; Rentzsch et al., 2007). Compared to the anthocyanins, pyranoanthocyanins have a maximum absorption wavelength in the region 495-520 nm, which presents a hypsochromic shift (Marquez et al., 2013; Rentzsch et al., 2007; Sáenz-Navajas et al., 2011).

The formation of pyranoanthocyanins by yeasts

The formation of vitisins

Vitisins are formed during wine fermentation by the condensation of anthocyanins present in the must and metabolites such as pyruvic acid (vitisin A-type compounds) or acetaldehyde (vitisin B-type compounds). The main anthocyanin present in the grape must, malvidin-3-*O*-glucoside reacts with pyruvic acid to form vitisin A and with acetaldehyde to form vitisin B (**Fig 2**), respectively (Escott et al., 2016; Suárez-Lepe & Morata, 2012). Although the most studied vitisins are vitisin A and vitisin B, vitisins can be also formed from other anthocyanins and their acylated derivatives (Morata et al. 2003a; Suárez-Lepe & Morata, 2012). The chromatic properties of vitisins differ from those of the grape anthocyanins. Maximum absorption for malvidin-3-*O*-glucoside in visible spectra is approximately 528 nm, however, vitisin A shows absorption maximum of 515 nm and vitisin B of 495 nm. Compared to the anthocyanins, vitisins are more resistant to oxidative damage due to the higher number of resonant forms present which results from the double pyranose ring structure. Additionally, they are less sensitive to SO_2 bleaching (Morata et al., 2016b).

During fermentation, glucose is transformed into pyruvate which is further metabolized into acetaldehyde that serves as a terminal electron acceptor in the production of ethanol. Some of the produced pyruvate and acetaldehyde diffuse out of the cytoplasm, thus providing precursor molecules for the formation of vitisins. The amount of pyruvic acid and acetaldehyde released during the fermentation varies with yeast strain. This criterion could be used in the yeast strain selection (Morata et al., 2003a; Suárez-Lepe & Morata, 2012). Additional factors influencing the formation of vitisins are pH of the wine and content of SO_2. To facilitate the formation of vitisins during fermentation, it is better to use small amounts of SO_2. Temperature is an additional factor that plays a role in the formation of vitisins. At 30°C the production of vitisin A and especially vitisin B can be reduced. Acetaldehyde is a very volatile compound and the rate of its evaporation during fermentation increases if the temperature is over 30°C (Suárez-Lepe & Morata, 2012).

Morata et al. (2003a) studied nine native and one commercial *S. cerevisiae* strains for the excretion of pyruvate and acetaldehyde during fermentation and the subsequent synthesis of vitisin A and vitisin B. The results showed that acetaldehyde concentration varies greatly within yeast strains as some of them were able to produce more than 120 mg/L of acetaldehyde during fermentation, while others produced less than 60 mg/L of acetaldehyde (Morata et al., 2003a). The average maximum concentration of pyruvate was 98 mg/L (Morata et al., 2003a). Acetaldehyde is found in wines in the concentration range 10-300 mg/L, and the content varies with the type of yeast strain used (Lambrechts & Pretorius, 2000), which was also observed by Morata et al. (2003a). Similarly, the content of pyruvic acid found in wines also varies with the type of yeast strain used. Chidi et al. (2015) tested five *S. cerevisiae* commercial strains (EC1118, DV10, BM45, VIN13, 285) and under aerobic conditions they produced from 535 mg/L to 1249 mg/L of pyruvic acid (Chidi et al., 2015), showing that the release of pyruvic acid during fermentation is affected by the yeast strain used and can vary greatly. Morata et al. (2003a) showed that *S. cerevisiae* strains that produced and excreted more pyruvic acid produced higher concentrations of vitisin A in the red wine fermentation. This was also observed for acetaldehyde, as yeast strains that produced more acetaldehyde synthesized higher values of vitisin B.

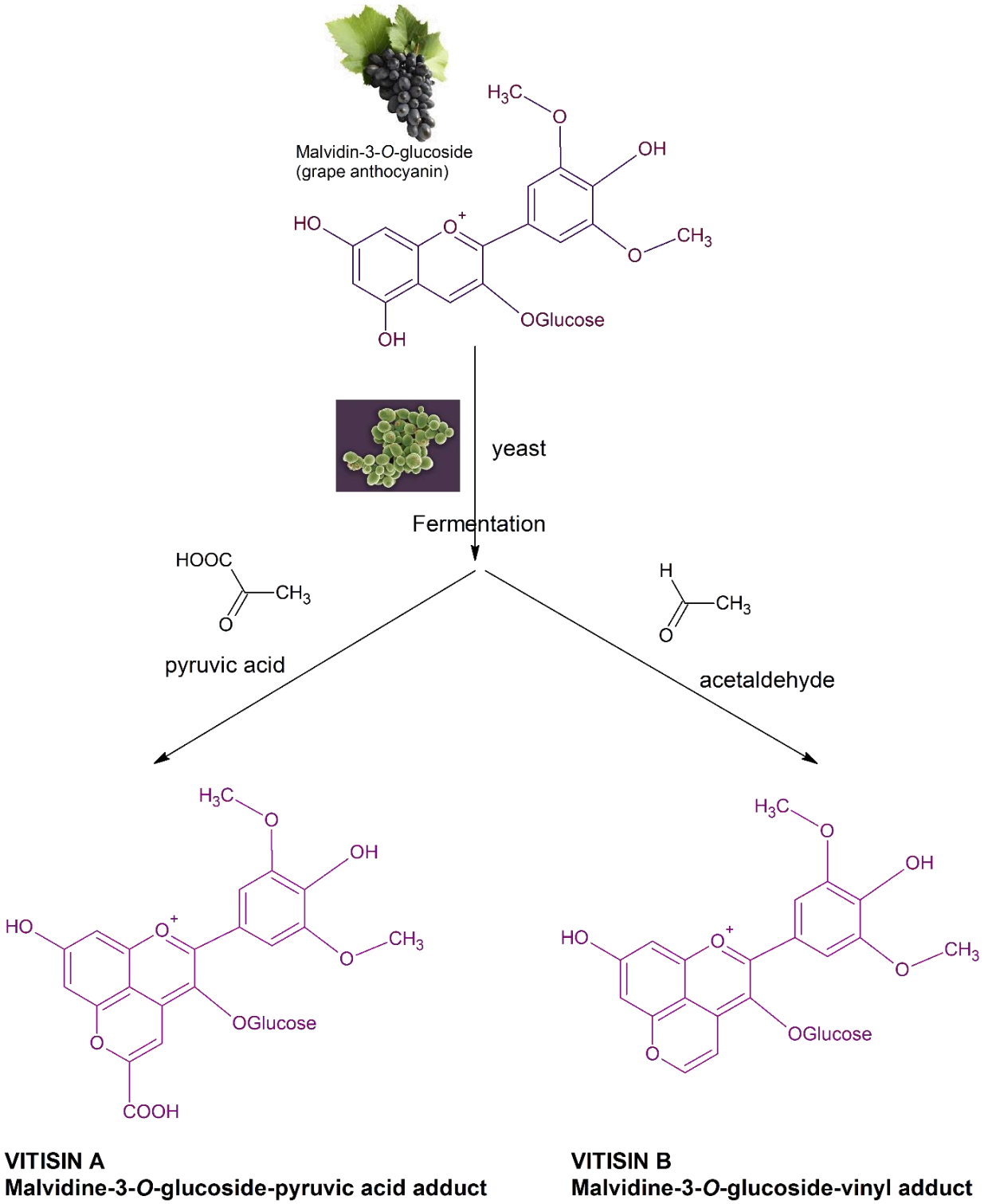


Fig 2. Synthesis of vitisins by reaction of two yeast metabolites released during fermentation (pyruvic acid and acetaldehyde) with malvidin-3-*O*-glucoside. Pyruvic acid and malvidin-3-*O*-glucoside yield vitisin A, acetaldehyde and malvidin-3-*O*-glucoside yield vitisin B.

The amounts of vitisin A and vitisin B formed were proportional to the releases of pyruvic acid and acetaldehyde, although the formation of vitisin A depended also on the metabolic differences between the tested strains (Morata et al., 2003a). Hayasaka et al. (2007) tested the effect of *S. cerevisiae* and *S. bayanus* yeasts on the colour and pigment profile of Cabernet Sauvignon. The results showed that the wine

fermented with *S. bayanus* exhibited higher values of wine colour density (sum of absorbance at 420 nm and 520 nm) and higher values of SO_2 non-bleachable pigments. The wine fermented with *S. bayanus* synthesized acetaldehyde-mediated pigments (i.e. vitisin B) in higher concentrations compared to *S. cerevisiae* strain tested, which could be a result of the difference in the acetaldehyde production between two strains. The authors reported that even though the levels of acetaldehyde-mediated pigments decreased during storage (more than one year), the wine fermented with *S. bayanus* maintained higher values of colour density, pigmented polymers, and SO_2 non-bleachable pigments. The results suggest that acetaldehyde-mediated pigments could act as precursors for more stable pigmented polymers. The research showed that the use of two different yeasts can result in the differences in the colour properties of wine (Hayasaka et al., 2007).

Medina et al. (2016) tested six non-*Saccharomyces* wine yeasts belonging to the *Metschnikowia (M. pulcherrima)* and *Hanseniaspora (H. guilliermondii, H. opuntiae, H. vineae, H. clermontiae)* genera using pure and mixed cultures with *S. cerevisiae* Sc882 strain for their effect on red wine colour using artificial red grape juice medium. All tested strains produced vitisin B, with *S. cerevisiae* Sc882 strain producing the highest value. This could be attributed to higher acetaldehyde levels produced by *S. cerevisiae* strain compared to non-*Saccharomyces* yeasts. Results also showed that the co-inoculation of *S. cerevisiae* strain with *Metschnikowia* or *Hanseniaspora* strains resulted in the significantly increased production of vitisin B compared to the pure culture fermentation (Medina et al. 2016). Loira et al. (2015) tested three *S. pombe* and three *T. delbrueckii* yeast strains in co-inoculated and sequentially inoculated fermentations with *S. cerevisiae* yeast. *S. pombe* strains showed a greater synthesis of vitisins in sequential fermentations, while *T. delbrueckii* synthesized higher concentrations of vitisins in the mixed fermentations (Loira et al., 2015).

Medina et al. (2018) evaluated 49 strains belonging to 12 species for their effect on wine colour (colour intensity, hue, total anthocyanins, and total polyphenol index). Six selected strains from the genus *Hanseniaspora (H. guilliermondii Hg T06/09G; H. opuntiae Ho T06/01G; H. vineae TO2/05F; H. clermontiae C10/54F and A10/82F)* and *Metschnikowia (M. pulcherrima M00/09G)* and one *S. cerevisae* Sc882strain were further tested for vitisin A and vitisin B formation. All seven strains showed formation of vitisin B, however *S. cerevisae* strain produced two times higher concentration in comparison to the best non-*Saccharomyces* strain. With vitisin A, the trend was different as higher levels of vitisin A were found in fermentations with non-*Saccharomyces* yeast compared to the *S. cerevisae* yeast tested (Medina et al., 2018).

The Formation of vinylphenolic pyranoanthocyanins

Vinylphenolic pyranoanthocyanins are condensation products between vinylphenols and anthocyanins. The first vinylphenolic pyranoanthocyanin structure was identified in the late 1990s as pyranomalvidin-3-glucoside-phenol. It was identified in red wine after extraction from polymeric membranes during cross-flow microfiltration (Cameira dos Santos et al., 1996; Fulcrand et al., 1996). Afterwards, other similar structures were identified in wine but with different substitution patterns in the phenol fraction (catechol, syringol or guaiacol). In the cv. Pinotage pyranomalvidin-3-*O*-glucoside-catechol was identified and named Pinotin A. Pinotin A is formed by the reaction of malvidin-3-*O*-glucoside and caffeic acid (Schwarz et al., 2003a; 2004).

Yeast with high hydroxycinnamate decarboxylase activity (HCDC) can be used for decarboxylation of hydroxycinnamic acids into vinylphenols, that condense with anthocyanin and subsequently vinylphenolic pyranoanthocyanins are formed (**Fig 3**) (Suárez-Lepe & Morata, 2012). The reaction mechanism consists of decarboxylation of hydroxycinnamic acids into vinylphenol by enzyme hydroxycinnamate decarboxylase and reaction of vinylphenols with grape anthocyanins (Escott et al., 2016). The proposed mechanism involves a cycloaddition between the vinyl group of vinylphenol and the groups in position 4 and 5 of the anthocyanin, followed by oxidation resulting in aromatization of the D ring (Marquez et al., 2013). Hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic, sinapic acid) can also react directly with anthocyanins, but this takes place slowly, during ageing of wine (Hayasaka & Asenstorfer, 2002; Schwarz et al., 2003b).

Yeast strains can be tested for HCDC activity using a medium with added *p*-coumaric acid. The yeast is considered HCDC positive when it transforms more than 10% of *p*-coumaric acid. Greater transformation rate of *p*-coumaric acid results in the higher HCDC activity of the yeast. Subsequently, it is expected that vinylphenolic pyranoanthocyanins will be synthesized in higher concentration (Morata et al., 2016a). The presence of HCDC activity is quite common in *Saccharomyces* yeast and has been reported in non-*Saccharomyces* strains as well, however it is strain-dependent and the activity can vary greatly between species and strains (Benito et al., 2009; Kosel et al., 2014; Morata et al., 2013, Shinohara et al., 2000; Smit et al., 2003).

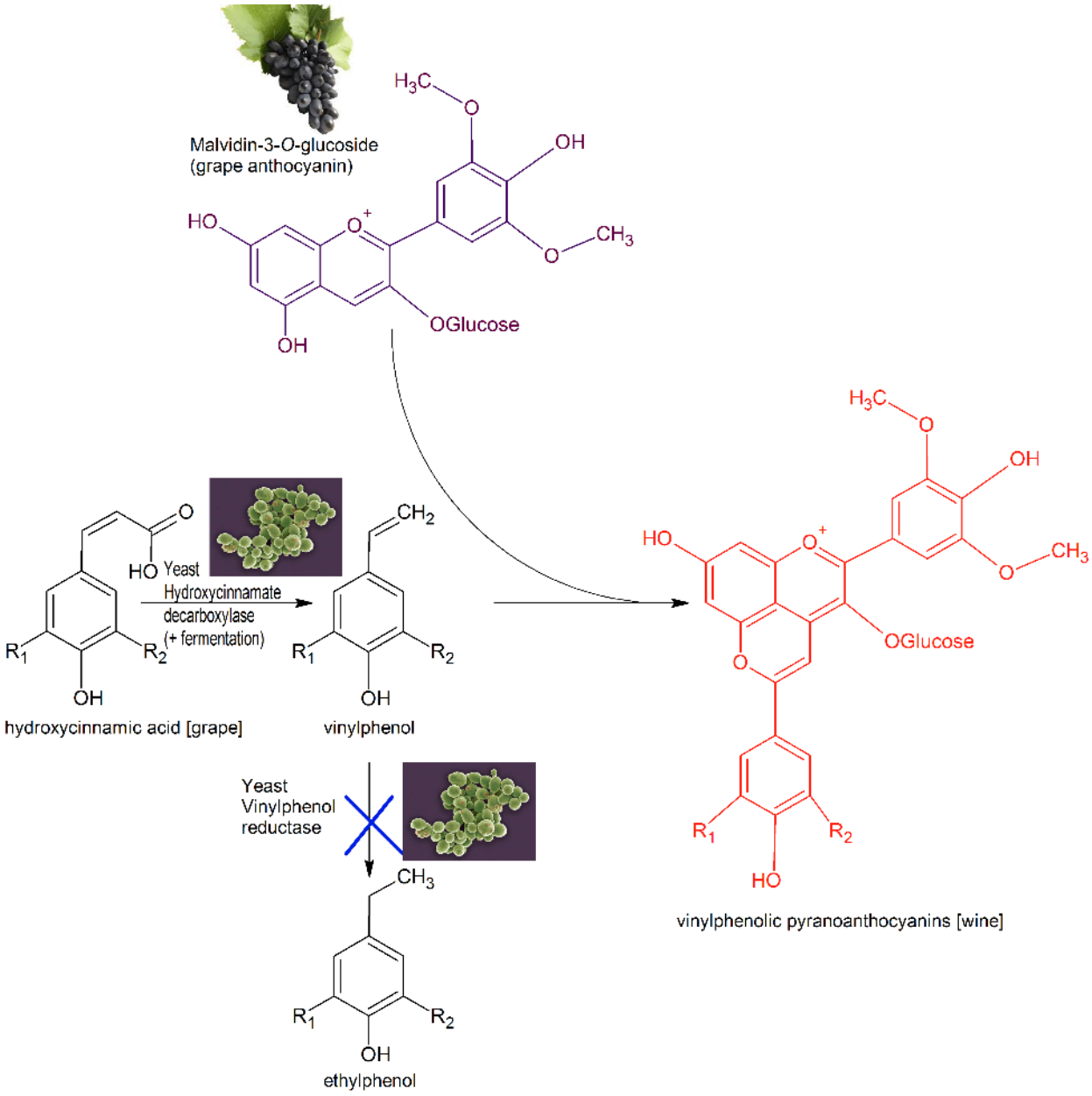


Fig 3. The formation of vinylphenolic pyranoanthocyanins through decarboxylation of precursor hydroxycinnamic acids by yeasts with HCDC enzyme activity. HCDC active yeasts decarboxylase hydroxycinnamic acids into reactive vinylphenols which react with anthocyanins and form vinylphenolic pyranoanthocyanins such as malvidin-3-*O*-glucoside-4-vinylphenol.

Morata et al. (2007) studied the formation of vinylphenolic pyranoanthocyanins in a Tempranillo must, a common grape variety from Spain. They supplemented must with various hydroxycinnamic acids and fermented it with different *Saccharomyces* strains. Vinylphenolic pyranoanthocyanins were produced when a sufficient amount of hydroxycinnamic acid was available in the must. The formation of these stable pigments was favoured when yeasts with HCDC activity were used as starters. Pigments were also formed in the must without the addition of hydroxycinnamic acids, and a difference in

the concentration was observed between the tested yeast strains. In the control sample, malvidin-3-*O*-glucoside-4-vinylguaiacol (adduct between malvidin-3-O-glucoside and ferulic acid/4-vinylguaiacol), was formed. The results suggest that the selection of yeast with proper HCDC activity could increase the content of stable pigments in wine, which would be of interest in the production of red wines that are to be aged for longer periods. When using yeasts that possess high HCDC activity and can also produce a high amount of vitisin A, bluish stable pigment vinylpyranomalvidin-3-*O*-glucoside could potentially be synthesized (Morata et al. 2007). This pigment is formed by the reaction of vitisin A and vinylphenol and could provide a potential source for natural blue food colorants (Mateus et al. 2004).

Benito et al. (2009) tested five HCDC active *S. cerevisiae* strains for the facilitation of vinylphenolic pyranoanthocyanins to reduce the content of hydroxycinnamic acids, thus preventing the formation of volatile phenols (4-ethylphenol, 4-ethylguaiacol) by *Dekkera/Brettanomyces* during wine ageing in barrels. The formation of vinylphenolic pyranoanthocyanins differed significantly among the tested strains, depending on the HCDC activity of strains. The maximum final values reached were around 8 mg/L (5% of the total anthocyanin content). The main vinylphenolic pyranoanthocyanin produced was malvidin-3-*O*-glucoside-4-vinylphenol. The difference in the production of vinylphenolic pyranoanthocyanins between tested strains reached 95%. The results showed that vinylphenolic pyranoanthocyanins were produced quicker before the stabilisation of the total anthocyanin content, which could be a result of the enrichment of fermentation medium with ethanol and the consequent loss of solubility of anthocyanins. This could be a limiting factor in the formation of vinylphenolic pyranoanthocyanin adducts (Benito et al., 2009). Recently Medina et al. (2018) tested six non-*Saccharomyces* species for vinylphenolic pyranoanthocyanin formation belonging to the *Metschnikowia* (*M. pulcherrima* M00/09G) and *Hanseniapora* (*H. guilliermondii* Hg T06/09G*; H. opuntiae* Ho T06/01G; *H. vineae* TO2/05F; *H. clermontiae* C10/54F and A10/82F) genera. All of the six tested strains showed the formation of malvidin-3-*O*-glucoside-4-vinylguaiacol, however, there was no statistical difference among them. Furthermore, all five of the tested *Hanseniaspora* species also synthesized malvidin-3-*O*-glucoside-4-vinylphenol. *M. pulcherrima* MP M00/09G was an exception, as it did not synthesize this pigment. However, it produced the highest amount of malvidin-3-*O*-glucoside-4-vinylguaiacol, suggesting more specific HCDC activity for ferulic acid than *p*-coumaric acid (Medina et al. 2018).

Anthocyanin adsorption on cell walls of yeast

The composition and porosity of fermenting yeast cell walls can cause significant losses of must aroma and colour via adsorption of the volatile compounds and the adsorption of anthocyanins to the cell walls, respectively. The latter property is especially important in grape varieties that have lower anthocyanin contents such as Pinot Noir (Morata et al., 2005). A survey of more than 170 commercially available wines showed that Pinot Noir had the lowest anthocyanin concentration (60 mg/L), with Merlot and Cabernet Sauvignon having twice higher concentrations of anthocyanins (Cliff et al. 2007). Mazza et al. (1999) tested the influence of several commercial *S. cerevisiae* yeast strains on the phenolic composition and colour of 1996 and 1997 Pinot Noir, Merlot, and Cabernet Franc wines. The yeasts used for fermentation did not affect wines noticeably, except for Pinot Noir wines. In the case of 1997 Pinot Noir wines, fermentation with *S. cerevisiae* Wädenswil 27 strain gave lower colour density, total phenolic content and total anthocyanin content in comparison to the other two *S. cerevisiae* tested strains (RC212 and Ra17). The fermentation kinetic of *S. cerevisiae* Wädenswil 27 strain was slower in comparison to RC212 and Ra17 *S. cerevisiae* strains, which could have led to the differences (Mazza et al., 1999). The anthocyanin adsorption capacity of yeast had not been investigated but could also be a potential reason behind the lower anthocyanin contents.

There are two main approaches in the assessment of yeast ability to adsorb anthocyanins on the cell wall – qualitative and quantitative (Morata et al., 2016). The qualitative technique is based on the visual analysis of growing yeast colonies in a solid medium enriched with grape anthocyanins such as yeast-peptone-dextrose (YEPD) medium. Caridi et al. (2013) measured adsorption of grape skin pigments on yeast YPD medium with the addition of citric acid monohydrate and disodium hydrogen phosphate with pH of the medium optimized to 3.5 to mimic the wine pH. After inoculating the yeast on plates and incubation the biomass was collected and spread on a flat surface for photographing. The colour assessment was performed using photographs of yeast, measuring their red, green and blue components with the Adobe Photoshop program. The tested yeasts showed significant differences in their colour components (Caridi, 2013; Caridi et al., 2015). The developed method provides useful and inexpensive tool to measure the adsorption of grape skin pigments and

could be used to distinguish yeast strains with high adsorbing capacity and low adsorbing capacity.

Echeverrigaray et al. (2019a) used 96 well microtiter Elisa-plates for determination of yeasts' ability to adsorb pigments and for the evaluation of wine colour intensity and tonality. They evaluated 22 commercial *S. cerevisiae* wine yeast strains and three *S. cerevisiae* strains isolated from Brazilian vineyard (MPF, LACF, LACF). They used three musts (two *Vitis vinifera* L. cv. Cabernet Sauvignon and Merlot and one *V. labrusca* cv. Ives) for determination of yeasts' ability to adsorb pigments. The results showed significant variation between tested *S. cerevisiae* strains in the anthocyanin adsorption. The grape varieties had not had any impact on cell wall adsorption properties since the strains that were grouped as low, medium or high adsorption capacity strains, behaved similarly among all three grape varieties tested (Echeverrigaray et al., 2019a).

The second approach is quantitative and provides additional information such as the concentration of anthocyanins adsorbed on the yeast cell wall, their structures and the ratio between different adsorbed pigments. Morata et al. (2016a) inoculated fresh red must with five *S. cerevisiae* yeasts (Uvaferm™ HPS™, Uvaferm™ VRB™, LALVI™ CLOS™, 3VA, 7VA) and one *S. cerevisiae x S. bayanus* yeast (Lalvin™ S6U™). After the fermentation, the anthocyanins were extracted from lees with 10% formic acid in methanol. The supernatants containing adsorbed anthocyanins were analysed using HPLC-DAD. The adsorption percentages of anthocyanins were defined as the ratio between their concentration in the wine and the cell walls. The ability of the tested yeasts to adsorb anthocyanins ranged from 9.4 – 11.9%. The variation in the adsorption of anthocyanins was attributed to the differences in the composition and the structure of their cell walls (Morata et al., 2016). The cell wall of *S. cerevisiae* strains is made of mannoproteins that are bound to oligopolysaccharides. Anthocyanin-binding molecules are localized in the inner part of yeast cell walls and different polarities of these wall polymers define the capacity of yeast to adsorb pigments such as anthocyanins (Echeverrigaray, et al., 2019a,2019b; Fernandes et al. 2017; Morata et al., 2003b, 2005). The majority of the publications about anthocyanin adsorption capability of yeasts is on *S. cerevisiae* strains. Further research that would include also non-*Saccharomyces* strains is needed to determine if some yeast genera or yeast species adsorb more anthocyanins than the others.

Conclusion

The wine industry is constantly searching for yeast strains that could result in the production of wine with better sensory and colour properties. However, when selecting yeasts, one should take into account that possible enhancement of one wine characteristic could have a detrimental effect on the other wine properties. In lieu of this, the potential wine starters should be characterized fully, and their effect on colour and aroma should always be characterized. Additionally, utilization of mixed starters should also be taken into account, as they can result in better organoleptic characteristics of the wine in comparison to the usage of single wine starters. The grape microbiome is complex and vast and undoubtedly presents new opportunities for exploration in wine production. One of the main reasons for using non-*Saccharomyces* strains in the production of wine is also to produce wines that would reflect the wine region. Selected *Saccharomyces* and non-*Saccharomyces* strains can enhance primary and secondary wine aroma and can be involved in the control of the spoilage wine microflora, the release of mannoproteins and the reduction of the ethanol content of the wine. Furthermore, the selection of specific yeast strains can result in colour stabilization and synthesis of more stable pigments.

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