Functional Metabolism of Aromatic Precursors in *Hanseniaspora*: A Source of Natural Bioactive Compounds.

Maria Jose Valera*[1,2]*, Eduardo Boido*[1]*, Laura Fariña*[2,3]*, Eduardo Dellacassa*[3] *and Francisco Carrau* [1,2]*.

[1] Enology and Fermentation Biotechnology Area, Department of Science and Food Technology. Faculty of Chemistry, Universidad de la Republica. Montevideo, Uruguay. [2] PAYS. Programa de Alimentos y Salud, Faculty of Medicine, Universidad de la República, Montevideo Uruguay. [3] Laboratorio de Biotecnologia de Aromas, Faculty of Chemistry, Universidad de la República, Montevideo Uruguay.
*fcarrau@fq.edu.uy

Abstract

Hanseniaspora species are among the most prevalent yeasts found on grapes and other fruits, with a growing role in wine fermentation due to their distinctive metabolic profiles. This review focuses on the functional divergence within the genus, particularly between the fast-evolving fruit clade and the slow-evolving fermentation clade. While species in the fruit clade often exhibit limited fermentation capacity with interesting enzymatic activity, members of the fermentation clade—especially *H. vineae*—demonstrate moderate fermentative potential and a unique ability to enhance acetylated aromatic alcohols with healthy properties. When used in mixed fermentations with *Saccharomyces cerevisiae*, some *Hanseniaspora* species contribute significantly to the production of bioactive and aromatic compounds, including tyrosol and tryptophol, and their acetate esters, benzenoids, melatonin and other derived compounds with functional properties. The metabolic activity of *Hanseniaspora* is also marked by robust extracellular enzymatic functions and a rapid autolytic profile, facilitating the release of aroma precursors and phenolic compounds. This review emphasizes the role of aromatic amino acid-derived pathways—namely the shikimate, phenylpyruvate, mandelate, and Ehrlich routes—in the biosynthesis of aroma-active metabolites. Overall, *Hanseniaspora* species represent promising non-*Saccharomyces* yeasts for modulating wine aroma and composition, with implications for both industrial fermentation strategies and fundamental yeast biology.

Key words
Wine yeast, flavor compounds, fermented foods, shikimate pathway, tyrosine, tryptophan, phenylalanine.

Introduction

Yeasts of the genus *Hanseniaspora* are the most frequently isolated in grapes and other fruits. It is common for these species to appear at the beginning of spontaneous fermentations (as apiculate or lemon shape cells), and then they are generally displaced by *Saccharomyces* strains (Romano et al. 2003, Medina et al. 2013, Lleixa et al. 2016). Currently, 23 species of *Hanseniaspora* are recognised (van Wyk et al., 2024), of which approximately ten have been found to be associated with grapes and wine. It has been determined that certain taxa exhibit substandard fermentation capabilities, thus characterising them as the fast-evolving fruit clade (Valera et al., 2021). Conversely, other species of this genus have been identified as the slow-evolving fermentation clade. In the fruit group, the following species were identified in grapes: *Hanseniaspora valbyensis, H. guilliermondii, H. uvarum, H. opuntiae, H. thailandica, H. meyeri, and H. clermontiae.* The term "fast evolving" is employed to denote that these species are characterised as being highly unstable in terms of their genetic variability. In the fermentation group, the following species are present: *Hanseniaspora vineae, H. osmophila, H. occidentalis, H. taiwanica* and *H. gamundiae.* These species exhibit slow evolution in their genome due to their higher stability genome compared to the fruit group (Steenwyk et al., 2019). These genetic differences also have a phenotypic expression, with respect to their fermentative capacity and aroma production (Valera et al., 2021; Martin et al., 2018).

For many years, this genus of yeasts was regarded as a group of microorganisms that were not desired for fermentation purposes. This is particularly pertinent given that they are regarded as increasing volatile acidity (Ciani and Picciotti, 1995). However, this attribute is strain-dependent, and certain species have been identified as prominent producers of acetic acid, notably those classified within the fruit group. However, it is noteworthy that certain strains of *Hanseniaspora vineae* have demonstrated comparable or diminished levels of volatile acidity production in comparison to those observed in *Saccharomyces cerevisiae* under analogous fermentation conditions (Del Fresno et al., 2021; Medina et al., 2013).

Although the fact that the fermentative capacity of the *Hanseniaspora* fermentation group is significantly superior to that of the fruit group, it is nevertheless regarded as moderate in comparison to *Saccharomyces* species. Consequently, the most efficacious approach for the utilisation of the active dry yeast *H. vineae* (Fermivin® VINEAE, Oenobrands) at an industrial level is through the implementation of mixed cultures. This strategy is enabling winemakers to exploit the aromatic potential of certain *Hanseniaspora* strains, thereby contributing to enhance aromatic diversity in the final wines. In practice, the co-inoculation of *Saccharomyces* in low proportion (10-20% of the initial inoculum) resulted in a fermentation rate that was comparable to that of pure cultures of *Saccharomyces,* and furthermore, the flavour impact was obtained (Gallo et al., 2024). Figure 1 shows *Hanseniaspora* cells under an electron microscope. As their shapes differ so much from those of *Saccharomyces* cells, a simple optical microscope is sufficient for monitoring the fermentation proportions of mixed cultures at wineries.

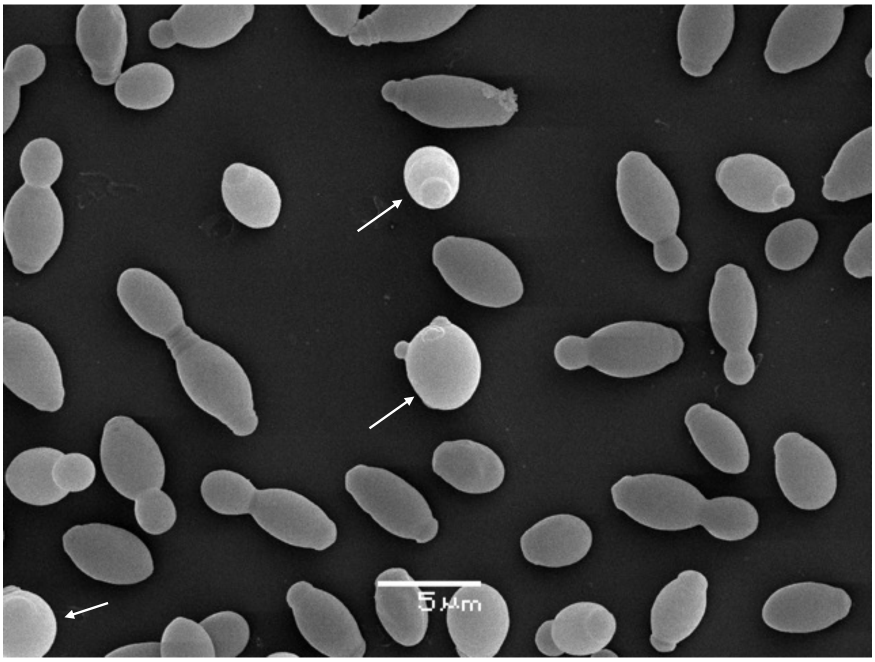

Figure 1: *Hanseniaspora vineae/Saccharomyces* mixed cells (proportions 90/10%) fermenting grape juice. While *S. cerevisiae* exhibits a well-known multipolar budding process, leaving scars on the surface of the round cell with each division (white arrows show 3 cells of *S. cerevisiae*), *Hanseniaspora* species exhibit superimposed scars described as concentric funnels with parallel rims at each pole. Each scar indicates the birth of a daughter cell; in this image, for example, an older cell has formed three daughters, born from the upper and lower poles (six daughters have been born in total). Younger cells around the larger cell acquire a more typical apiculate (lemon-shaped) form as they increase in number along the long axis. Younger *Hanseniaspora* cells are shown at the bottom of the figure with just one scar on one of the poles. They are preparing to start cell division at the opposite pole, which they will then do alternately from each pole during exponential phase. Scanning electron microscope photography from the Carrau Laboratory.

The employment of mixed cultures of *Hanseniaspora* and *Saccharomyces*, in addition to enhancing the aromatic profile, confers numerous advantages. Several factors must be considered, including the increase in bioactive compounds, such as the higher production of phenylethyl acetate levels due to the involvement of *Saccharomyces* that contributes with extra alcohol precursor synthesis (Zhang et al. 2020; Carrau and Henschke, 2021), and a decrease in ethanol content, the modulation of acid production and the increase in anthocyanin-derived compounds. Furthermore, the proteolytic capacity of certain strains has been demonstrated to reduce the need of bentonite addition for the protein stabilization of white wines (Martin et al., 2022; Carrau et al., 2023; van Wyk et al., 2024). *Hanseniaspora* species are producers of many other extracellular enzymes that might contribute to the final composition of foods depending on the fermented substrate (Lopez et al. 2015).

The fermentation group of the *Hanseniaspora* genus exhibits reduced efficiency in the production of ethanol when compared with *Saccharomyces.* This reduced efficiency is accompanied by an increased activity in secondary metabolic pathways. Many metabolic pathways are involved in the production of bioactive compounds in *Hanseniaspora*, including those derived from the mevalonate pathway. Some sesquiterpenes have been observed to be produced in higher quantities by *H. vineae* than *Saccharomyces* (Martin et al., 2022), and among monoterpenes, there has been an interesting production of some rare compounds, such as safranal, which was detected at concentrations above its sensory threshold (Del Fresno et al., 2022). This compound is the primary terpenoid of saffron stigmas, and it is regarded as the one of the most expensive food ingredients in the world. It has also been demonstrated to possess significant health-promoting properties (Nanda and Madan 2021). It is evident that several processes contribute to the final aroma of wine. For instance, the production of extracellular enzymes (mainly extracellular β-glucosidase in all the species of *Hanseniaspora* genus) allows the release of glycosidic aromatic precursors present in grapes, including alcohols such as benzyl and phenylethyl alcohols, or norisoprenoids, and other terpenoids. However, glucosidase enzymes not only increase the amount of volatile compounds but also modify phenolic compounds in grapes (Zhang et al. 2021). It has been reported that some strains of *H. uvarum* can increase the free (biologically active) form of resveratrol during fermentation (Gaensly et al. 2015). Furthermore, the accelerated cell lysis capacity exhibited by *H. vineae*, which occurs within days as opposed to the months required by *Saccharomyces* (Carrau et al., 2023), suggests the potential release of membrane and intracellular compounds that are associated with the aromas and texture of wine. These compounds may contribute to the final composition of wines. The present review will concentrate on bioactive compounds derived from the three aromatic amino acids that are part of the shikimate, phenylpyruvate, mandelate and Ehrlich pathways. These metabolic fluxes have been shown to be highly active in many species of *Hanseniaspora*, in comparison to conventional *Saccharomyces* strains.

Shikimate, phenylpyruvate and mandelate pathways

The shikimate pathway begins with the condensation of phosphoenolpyruvate and erythrose-4-phosphate to form shikimate (Fig. 2). From there, it proceeds through chorismate to produce prephenate or anthranilate. Chorismate is a key molecule, precursor of the biosynthesis of the three aromatic amino acids in yeasts: tyrosine, phenylalanine and tryptophan. The prephenate is converted in several steps to either phenylacetic acid or 4-hydroxyphenylacetic acid depending on the amino acid used for transamination (phenylalanine or tyrosine respectively) forming the phenylpyruvate pathway. Finally, the mandelate pathway comprises the conversion of mandelate derived from phenylacetate or the 4-hydroxymandelate from 4-hydroxyphenylacetate to benzyl alcohol or 4-hydroxybenzyl alcohol respectively (Valera et al., 2020). The production of benzyl alcohol is significantly increased in *H. vineae* (Martin et al., 2016a). This characteristic allowed to describe the metabolic route to produce these compounds in yeast, including 4-hydroxybenzaldehyde, a precursor of Coenzyme Q6 (CoQ6) benzenoid core. This pathway was confirmed to be similar in *Saccharomyces* (Valera et al., 2020), but the concentrations of the precursors analysed was very low compared with those quantified in *H. vineae*. In fact, *H. vineae* have the capacity to *de novo* synthesize benzenoid compounds such as benzyl alcohol, benzaldehyde, p-hydroxybenzaldehyde, and p-hydroxybenzyl alcohol in the absence of grape-derived precursors. This capability is attributed to the mandelate pathway, an alternative to the phenylalanine ammonia-lyase (PAL) pathway found in plants and basidiomycetes fungi. The mandelate pathway involves the conversion of phenylalanine to phenylpyruvate, then to benzoylformate, and finally to benzyl alcohol (Valera et al. 2021). The esterification of benzyl alcohol in *H. vineae* produces benzyl acetate, which possesses a jasmine-like aroma. Its presence contributes to the floral character of wines fermented with this yeast being a species marker as it is not found in *Saccharomyces* fermented products (Martin et al. 2016a). Genomic analyses have revealed the presence of an increased copy number (CN) of genes such as *ARO8*, *ARO9* (aromatic amino acid aminotransferases), and *ARO10* (phenylpyruvate decarboxylase) in *H. vineae*, which are involved in the catabolism of aromatic amino acids leading to the production of aroma compounds (Valera et al. 2020). It is interesting to note that the production of benzenoid compounds by *H. vineae* is influenced by the concentration of yeast assimilable nitrogen (YAN) in the must. As demonstrated by Martin et al. (2016b), lower YAN can result in increased production of these aroma compounds. These reactions are practically absent in *Saccharomyces* species but improved in a fermentation medium with very low assimilable nitrogen levels (Valera et al. 2020). This suggests that effective nitrogen management is essential for optimizing the increase in production of benzenoids. The concentration of these bioactive compounds can be enhanced through yeast by elucidating and optimizing metabolic pathways and fermentation conditions. In terms of genomics, it was found that the *Hanseniaspora* genus has similar gene copy numbers compared to *Saccharomyces* at the shikimate pathway and the formation of chorismate (Fig. 2B). An increase of CN in the following steps of the phenylpyruvate and mandelate pathways might explain the significant increased formation of benzenoids, tyrosine, tryptophane and phenylalanine, and many bioactive derived compounds. Nevertheless, further studies should be encouraged to explore other phenomena that may be increasing the flux through the shikimate pathway in *Hanseniaspora*. Such phenomena may include differences in gene expression and poor feedback inhibition of the *ARO3* and *ARO4* genes by the aromatic amino acids, as occur in plants (Almeida et al. 2024). Interestingly, the higher energy costs associated with synthesising the three aromatic amino acids, compared to linear amino acids, may explain the complex regulatory mechanisms employed by different cells to optimise these pathways. The relative costs of synthesising these were recently reported in *Saccharomyces* cells and found to be up to ten times higher than those of most other amino acids (Lemke et al., 2025).

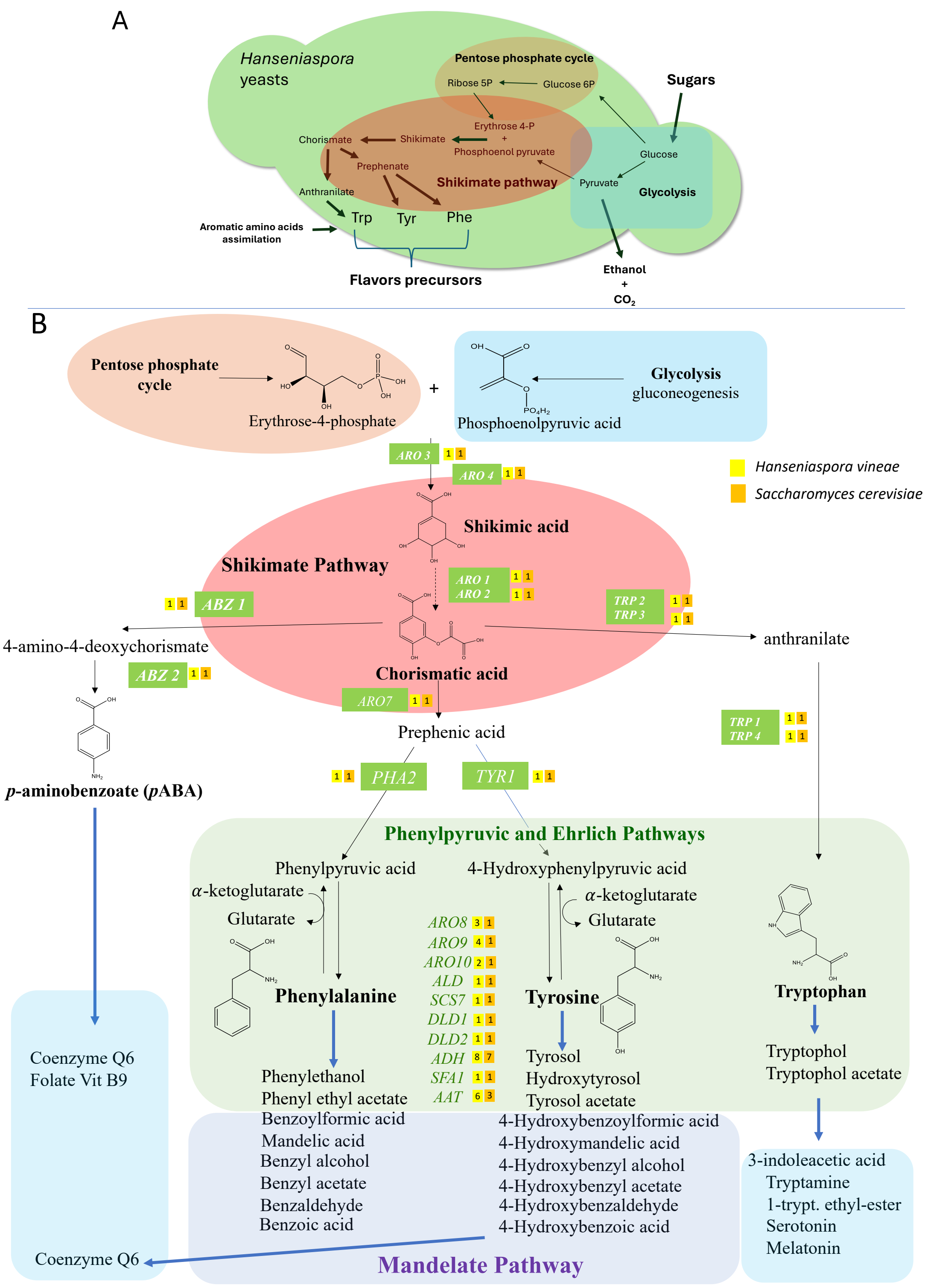

Figure 2. A. The shikimate pathway scheme shows the synthesis of the three aromatic amino acids that are precursor for the biosynthesis of flavors and bioactive compounds. This pathway is not present in animals, what explains their dependence on foods and their own gut microbiota to obtain many of these key bioactive compounds for nutrition. Trp, tryptophan; Tyr, tyrosine and Phe, Phenylalanine; Erythrose-4P, Erythrose-4-phosphate; Glucose-6P, Glucose 6-phosphate; Ribose-5P, Ribose-5-phosphate. B. Derived compounds of the shikimate and mandelate pathways. In orange the copy number (CN) of *Saccharomyces* genes that encode the enzymes involved in each conversion step and in yellow the CN in the *Hanseniaspora* genus genes. No differences in CN in the shikimate pathway were found in the *Hanseniaspora* species studied (*H.*

uvarum, H. osmophila, H. guilliermondii and *H. vineae*). The strong differences in CN were detected in the Phenylpyruvic, Ehrlich and Mandelate pathways (Valera et al. 2021).

Production of higher alcohols and their acetate esters from aromatic amino acids

Higher alcohols 2-phenylethanol, tyrosol and tryptophol derived from aromatic amino acids have been reported as bioactive compounds regulating yeast metabolism in *S. cerevisiae* (Avbelj et al., 2015) and in non-*Saccharomyces* species such as *H. uvarum* (Zupan et al., 2013). They are considered quorum sensing molecules, allowing cell density dependent signalization. For example, tryptophol modifies the growth rate and fermentation kinetics in non-*Saccharomyces* yeasts including *H. uvarum* (González et al., 2018; Valera et al., 2019).

The production of these alcohols with mixed cultures of *Hanseniaspora* and *Saccharomyces* varies significantly compared to wines produced with pure cultures of *Saccharomyces* (Olivera et al., 2024). This modulation due to the interaction of the two strains is most clearly observed by a significant increase in the acetate esters of higher alcohols, especially those produced from the higher alcohols derived from aromatic amino acids (phenylethanol, tryptophol and tyrosol). This acetylation capacity in *Hanseniaspora* allows the biosynthesis of acetate esters of these alcohols, a particular characteristic of this genus.

The production of esters takes place in four consecutive reactions (Fig 3A). First, an amino acid transamination involving proteins encoded by homologs of the genes *BAT1*, *ARO8* and *ARO9*. The species of the genus *Hanseniaspora* that have been analyzed do not present homologs of the gene *BAT2* that would participate in transamination in *S. cerevisiae,* but they do have several more copies of the *AROs* than *Saccharomyces* (Fig 2B). Then, the resulting α-ketoacids are decarboxylated by the action of the products of genes such as *PDC1* and *ARO10* giving rise to the corresponding aldehydes, which in turn produce higher alcohols by alcohol dehydrogenases such as those encoded by *ADH6*. These three reactions correspond to the Ehrlich mechanism and culminate in the production of higher alcohols (Fig. 3A). Finally, these compounds can be transformed into their acetates by the activity of alcohol acetyl transferases (AATs) that condense the alcohols with the carboxyl group of acetyl coenzyme A. Some strains of the genus *Hanseniaspora* are known to have large copy numbers of acetyl transferases-related genes that could explain the high rate of acetylation of higher alcohols detected in the extracellular medium in fermentations carried out by species of this genus.

The products of *ATF1* and *ATF2* genes synthesize volatile ester production in *S. cerevisiae*, however Atf1p was shown to be significantly more active in many *S. cerevisiae* strains (Verstrepen et al. 2003). In *S. cerevisiae* there are two essential regions in the products of *ATF* genes. The first is the WRLICLP, motif which is not strictly conserved in microorganisms (van Laere et al., 2008). The second is the H-X-X-X-X-D catalytic motif that appears in plant AATs. The catalytic residues His and Asp have been found to be crucial for AAT function being part of the active site in these enzymes (D'Auria 2006, Beekwilder et al., 2004).

In the case of *H. osmophila* and *H. vineae,* they present *ATF2* but not *ATF1* while *H. uvarum* does not present homologues of either (Valera et al., 2021). However, *H. uvarum* has two genes that encode different AATs (OEJ85955.1 and OEJ85967.1), which, despite lacking homology to *S. cerevisiae*, contains conserved domains and may represent functional analogues of the proteins encoded by the *ATF* genes. Recently, the first tools have been demonstrated being able to modify efficiently genes in *H. uvarum* (Badura et al., 2021; Heinisch et al., 2023). In these experiments, a mutant constructed of *H. uvarum* without the two copies of *ATF* showed a much lower production of acetate esters than the parental strain (Badura et al., 2021).

It has been shown that also products of homologous *SLI1* gene could perform this step in *H. vineae* (Giorello et al., 2019; Valera et al., 2021). In this species*, SLI1* gene copies are located in tandem within the genome. Each of them shows homology with a *SLI1* from *H. osmophila.* However, the *H. uvarum ATF* genes fall out of this cluster.

H. guilliermondii UTAD222 has 4 genes encoding AATs that are differentially expressed throughout fermentation. Moreover, according to Seixas et al. (2023) this expression is modulated by the carbon-nitrogen balance in the medium, resulting that lower carbon to nitrogen ratio increases acetyl transferase activity in the medium, generating more acetates.

Recently, tyrosol and tryptophol acetates have been also reported as interesting bioactive compounds in mammals metabolism. Both compounds exhibited hyperinflammation reduction capacity in murine models (Malka et al., 2023) and tryptophol acetate demonstrated a quorum sensing blocking activity in several pathogen bacteria, especially *Vibrio cholerae* (Malka et al., 2023). Tyrosol and tryptophol acetates are more liposoluble while alcohols are highly metabolized in gut and liver and they are not well absorbed (Rodriguez-Morató et al., 2016) therefore, the biodisponibility of ester acetates might be higher than their corresponding alcohols.

Neither *S. cerevisiae* nor *H. uvarum* and *H. osmophila* can produce these two acetate esters during the fermentation process (Fig. 3B) (Valera et al., 2021). Interestingly, different strains of *H. vineae* present similar production of tryptophol and tyrosol acetates during Chardonnay wine fermentation (Valera et al., 2024) (Fig. 3B). The explanation might be a specific AAT activity for these two alcohols in *H. vineae* which is not conserved among the genus *Hanseniaspora*.

Another derived molecule of tyrosol with bioactivity is hydroxytyrosol. This molecule, commonly found in olive oil, exhibits strong antioxidant properties and proven biological activity in humans. (Dudley et al., 2008). It is synthesized by *S. cerevisiae* during alcoholic fermentation in wine (Álvarez-Fernández et al., 2018) but it was not tested in fermentations performed by *Hanseniaspora* strains.

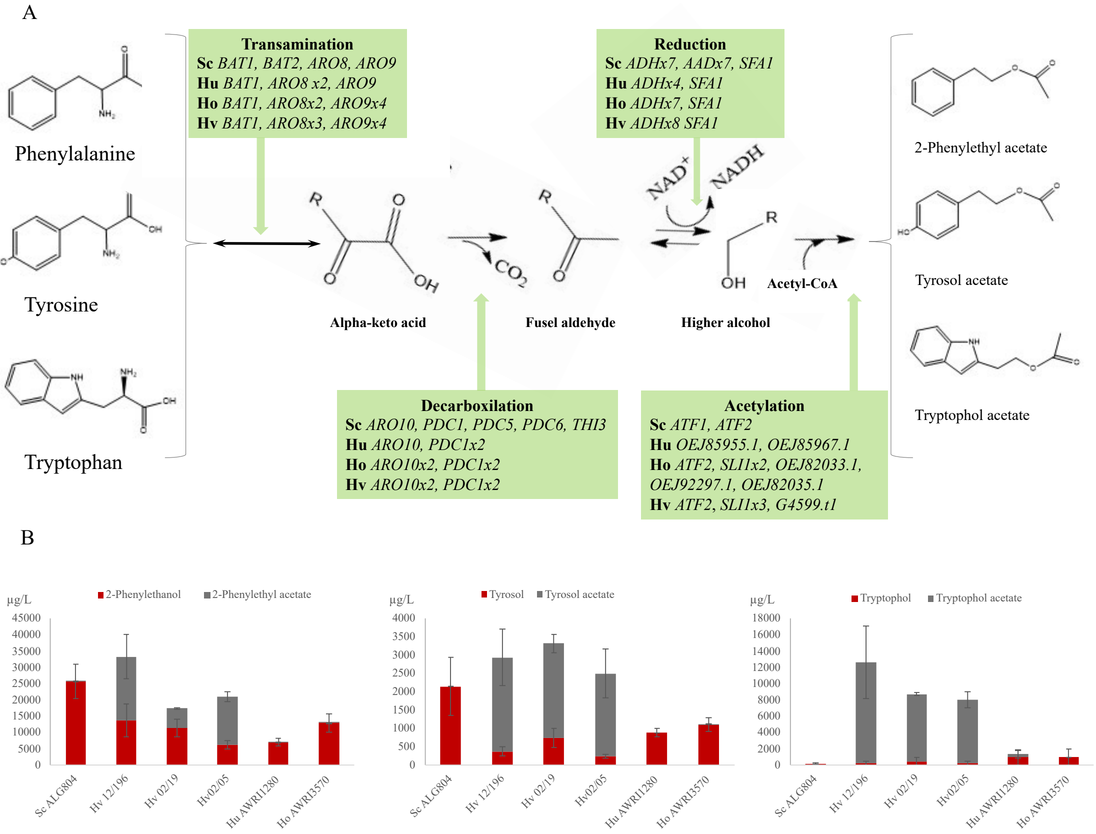

Figure 3. Production of acetate esters in yeast from the three aromatic amino acids: phenylalanine, tyrosine and tryptophan. A) Reactions involved in the formation of acetate esters and genes related to the formation steps of these compounds in different species of *Hanseniaspora* and *S. cerevisiae*. B) Production of acetate esters and higher alcohols derived from aromatic amino acids using different yeast strains of *Hanseniaspora* and after 10 days of fermentation in grape must. The commercial *Saccharomyces* wine strain ALG804 (PDM) was used as control (Valera et al., 2021; 2024). It is shown that the *Saccharomyces* strain cannot produce acetates from the three aromatic alcohols and not even produce tryptophol. **Sc** *Saccharomyces*, **Ho** *Hanseniaspora osmophila*, **Hu** *H. uvarum*, **Hv** *H. vineae*.

Indole metabolism and melatonin production

Depending on the redox state of the cell, aldehydes from the Erlich pathway can be alternatively oxidised to their corresponding acids, indole acetic acid, phenyl acetic acid, and 4-hydroxyphenylacetic acid (Mas et al., 2014). Indoleacetic acid is a bioactive molecule, a key phytohormone in plant metabolism, and it is also found in yeast fermentation in *S. cerevisiae* and *H. uvarum* (Fernandez-Cruz et al., 2019) which has been reported to have antioxidant properties (Boban et al., 2024).

Other tryptophan derivatives produced by yeasts have been described as bioactive compounds such as serotonin and melatonin. Serotonin is a neurohormone with an integral physiological role in the human body (Berger et al., 2009) and precursor of melatonin in vertebrates. It has been proposed as precursor of melatonin also in yeasts (Mas et al., 2014); as it was demonstrated with strain *S. cerevisiae* QA23, however, it has been not detected neither intracellular nor extracellularly in yeast fermentations by *H. uvarum* and other non-*Saccharomyces* strains (Fernandez-Cruz et al., 2019).

From serotonin, in yeasts melatonin can be synthesized via two alternative pathways: one involves N-acetylation to produce N-acetylserotonin, followed by O-methylation; the other way proceeds through O-methylation to form 5- methoxytryptamine, which is then N-acetylated (Muñiz-Calvo et al., 2019) (Fig 4A). Recent studies reveal the role of melatonin in yeasts as antioxidant (Vazquez et al., 2018; Sunyer-Figueres et al., 2020) improving fermentation kinetics of non-*Saccharomyces* strains (Valera et al., 2019) and acting as signal molecule related with the carbon balance during the fermentation (Morcillo-Parra et al., 2025). The production of melatonin is related to the fermentative capacity of the yeast strain. Higher fermentative strains of *Hanseniaspora* produce intracellularly more melatonin, especially in the lag phase (Fig 4B), compared to less fermentative strains (Morcillo-Parra et al., 2025).

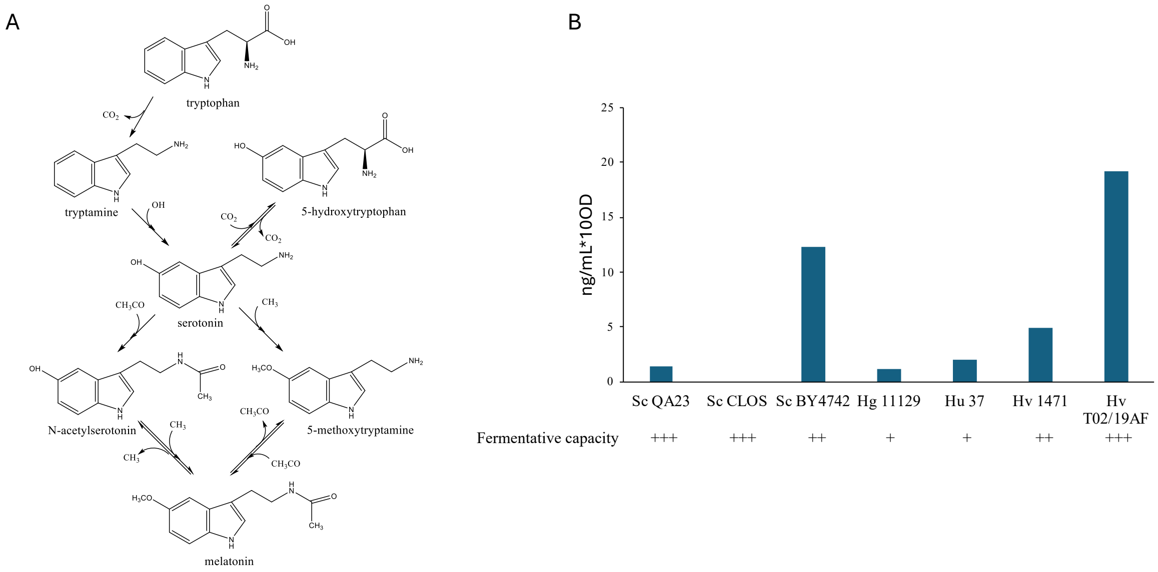

Figure 4. Production of melatonin. A) Proposed pathways of biosynthesis in yeasts B) concentration of melatonin detected in fermentative conditions during lag phase by different yeast strains with different fermentative capacity (data from Morcillo-Parra et al., 2025). **Sc** *Saccharomyces*, **Hg** *Hanseniaspora guilliermondii*, **Hu** *H. uvarum*, **Hv** *H. vineae*. There is a positive correlation between melatonin production and fermentation capacity in *Hanseniaspora vineae* strains. Further studies are necessary to confirm this with other strains of the genus.

Therefore, the highly fermenter *H. vineae* T02/19AF produced almost four times more melatonin than *H. vineae* 1471 with less fermentative capacity. And ten times more than *H. guilliermondii* 11129 and *H. uvarum* 37 (Morcillo-Parra et al., 2025). This behaviour was found by Jiao et al. (2022) who tested the production of melatonin of highly fermentative *H. uvarum* strains that dominated spontaneous wine fermentations.

In humans, melatonin is a neurohormone produced by the pineal gland controlling physiological processes, such as circadian rhythms and reproductive functions, also acting as an antioxidant (Serrano et al., 2010; Chahbouni et al., 2010; López et al., 2009).

Conclusion

Yeasts of the genus *Hanseniaspora* have very interesting properties due to their high production of distinctive aroma compounds for fermented foods. Studying these species will improve our understanding of the biosynthetic pathways of bioactive compounds derived from three aromatic amino acids: phenylalanine, tyrosine and tryptophan. These species contribute not only to the increased flavour complexity of fermented beverages, bread or chocolate, but also to the formation of bioactive compounds such as aromatic alcohols, acetates, benzenoids and their derivatives, including melatonin, serotonin and indole. The biotechnological advantages of this group of yeasts for the food industry will become apparent in the next few years.

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