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Minireview – Food & beverages

Genome-based exploration of volatile flavor diversity from food yeast species

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Abstract

Yeast shares a longer than 10 000-year history with humans in food fermentation by producing various volatile flavor compounds that contribute to the final taste and aroma of foods. Yeast-associated volatile flavor compounds include esters, benzenoids, sulfur compounds, and phenolic derivatives, which enhance the sensory complexity of fermented foods and beverages. Genome-scale technologies have advanced and transformed our understanding of the genetic and evolutionary drivers of volatile flavor diversity. The conventional approach to aroma enrichment and flavor balancing through single-strain optimization has been redefined through yeast cofermentation strategies, such as the pairing of *Saccharomyces cerevisiae* with nonconventional yeast species. This minireview summarizes the latest genomic insights into volatile flavor compound formation through ester, benzenoid, sulfur, and phenolic pathways in various yeast species and highlights the shaping of the next generation of food fermentation innovation via cofermentation combined with omics analysis, followed by a future perspective on synthetic biology for industrial applicability.

Keywords: volatile flavor compounds; genomics; *Saccharomyces cerevisiae*; nonconventional yeast; flavor diversity; yeast cofermentation

Introduction

Yeast is a eukaryotic microorganism that possesses the notable capability to produce a range of volatile flavor compounds that significantly influence the final taste and aroma of foods. Consequently, it shares a longer than 10 000-year history with humans in food fermentation. During evolution, survival- and reproduction-associated traits that directly enhance the fitness of the organism are more likely to be subjected to strong selective pressures. In general, flavor production-associated traits do not directly impact survival or reproduction, although acetate ester formation in *Saccharomyces cerevisiae* promotes the dispersal of yeast cells through insect vectors (Christiaens et al. 2014) and volatile compounds produced by *Metschnikowia reukaufii* attract oriental armyworm moths to pollen-rich yeast-fermented nectar (Ma et al. 2025). As a result, biosynthetic pathways in yeast species show variability over time, which enables a wide range of flavor diversity without negatively impacting their ability to thrive (Carrau et al. 2016).

Much of our understanding of the pathways related to the biosynthesis of volatile flavor compounds in yeasts has come from biochemical and molecular studies on the traditional yeast *S. cerevisiae*, the first eukaryotic organism to undergo whole genome sequencing in 1996 (Goffeau et al. 1996). However, in the last decade, a number of studies have reported the significant potential of non-*Saccharomyces* yeasts in aroma generation during fermentation, highlighting the importance of yeast diversity and the functional roles of emerging nonconventional strains in food fermentation (Varela 2016, Cao et al. 2024). Moreover, with the development of next-generation sequencing technology, the whole genome sequences of diverse nonconventional yeast species are currently available in public databases, allowing genomic information-based comparative genomics to investigate the genetic basis of flavor profiles in various yeast species and further facilitating functional genomics analysis based on various "omics" techniques. For example, a multi-omics study of the genome, transcriptome, and metabolome of *S. cerevisiae* and *Saccharomyces pastorianus* strains in ale and lager, the two main types of beer, has improved our understanding of the synthesis and regulatory mechanism associated with beer flavor compounds (Li et al. 2022). Genome-wide association study and weighted gene co-expression network analysis provided important information on genetic resources for their genetic improvement and creation of novel yeast strains for the production of new beer types (Li et al. 2022). Comparison of high-quality genome assemblies from >1000 natural *S. cerevisiae* isolates and related species showed that copy-number variation, accessory genes, and subtelomeric rearrangements contribute disproportionately to aroma phenotypes (Weller et al. 2023, Caudal et al. 2024). Quantitative trait loci (QTL) and genome-wide association studies have mapped dozens of loci with allele-specific effects on the production of esters such as ethyl octanoate and higher alcohols such as isoamyl alcohol (Eder et al. 2018, Gao et al. 2025). Pan-omics portals (e.g. ScRAPdb) now integrate these genotypes with transcriptomic, proteomic, and metabolomic data to enable researchers to trace volatile or-

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ganic compound (VOC) flux found in the headspace of fermented products from DNA sequences (Miao et al. 2025). Complementary systems biology resources have simultaneously increased in the postgenomic era. Yeast9 is a consensus genome-scale metabolic model for *S. cerevisiae* that links >2000 genes to 3000 reactions. This enables *in silico* prediction of VOC yields and guides strain design for ester enrichment or fusel-alcohol reduction (Zhang et al. 2024).

This minireview presents the latest research in volatile flavor diversity with functional analysis based on genome information of various food yeast species, focusing on esters, benzenoid, sulfur, and phenolic compounds. Additionally, recent strategies such as yeast cofermentation to enhance aroma complexity and balanced flavor profiles in food fermentation and future perspectives on synthetic biology of flavor-producing yeasts are presented.

Genetic features of biosynthetic pathways of volatile flavor compounds in yeasts

Key volatile flavor compounds produced by yeasts include esters, benzenoids, volatile sulfur compounds (VSCs), and phenolic derivatives. Each contributes distinctively to the aromatic complexity of fermented products (Table 1). To date, biosynthetic pathways and underlying mechanisms pertaining to flavor compounds have been elucidated based on conventional biochemical and molecular biological approaches, especially for *S. cerevisiae*. The increasing number of studies on the analysis of flavor formation by nonconventional yeasts, particularly when combined with genomic analysis, provides further advanced information on evolutionarily conserved and diverged biosynthetic pathways. Moreover, whole-genome sequencing of food-related yeasts enables the identification of genes linked to key traits, such as flavor production, thereby facilitating the development of more efficient and desirable strains for industrial applications. The number of yeast species with available genome data is steadily increasing, and key genomic features of aroma-producing strains are summarized in Table 2. Notably, comparative genomic analysis of several yeast species isolated from fermented foods, such as Korean traditional fermented alcoholic beverages and soybean products, has shown that inter-/sub-species hybridization between different yeast species or within the same species, resulting in the generation of heterozygous diploid genomes, occurs frequently as an evolutionary strategy in the fermentation environment (Jeong et al. 2023, Son et al. 2023). Diploids, in particular, provide genetic robustness that could buffer against harmful mutation, enhancing survival upon encountering harsh fermentation conditions, such as high sugar, ethanol accumulation, fluctuating pH, and nutrient depletion. In addition, diploids with extra gene copies can increase expression of stress-response genes, conferring higher stress tolerance. Moreover, heterozygous diploids with different alleles may complement each other's functions, improving performance.

This section describes the conserved and diverged biosynthetic pathways for each compound class, with a focus on their metabolic origins, key enzymatic steps, and relevance to yeast physiology and aroma production and summarizes recent advances in the elucidation of genomic features underlying volatile flavor production in *S. cerevisiae* and nonconventional yeast species. Particular attention is given to key genes involved in the biosynthesis of esters, benzenoids, sulfur-containing volatiles, and phenolic derivatives, which have been functionally validated through molecular biology and biochemical studies or identified via *in silico* analyses of yeast genomic sequences.

Genetic diversity in volatile ester biosynthesis

Volatile esters represent the most significant group of yeast-derived aroma-active compounds, as they impart highly desired fruity and floral notes to fermented food even in trace amounts (Saerens et al. 2010). Volatile esters are grouped into two major categories: acetate esters and medium-chain fatty acid (MCFA) ethyl esters. Acetate esters include ethyl acetate (solvent-like aroma), butyl acetate (sweet, banana), isobutyl acetate (fruity, banana), isoamyl acetate (banana), and phenethyl acetate (2-phenylethyl acetate; rose aroma). These compounds are synthesized by the condensation of acetyl-coenzyme A (acetyl-CoA), derived from glycolysis, with ethanol or with higher alcohols that originate from amino acid catabolism via the Ehrlich pathway (Fig. 1A). Large quantities of ethyl acetate are produced in the mitochondria, and >50% is produced via the condensation of ethanol and acetyl-CoA. The latter is primarily generated from the oxidation of pyruvate via pyruvate dehydrogenase (Pdh) in the mitochondria (Kruis et al. 2018a). Acetate esters easily diffuse from the cytoplasm to the extracellular environment owing to their small size and lipophilic characteristics; in contrast, the efficiency of MCFA ethyl esters in penetrating the membrane decreases depending on their hydrocarbon chain length. Consequently, acetate esters with lower sensory threshold concentrations and higher volatile capacity exert a much greater impact on flavor and fragrance than their fatty acid counterparts (Saerens et al. 2010, Dzialo et al. 2017).

Acetate ester biosynthesis in *S. cerevisiae* is catalyzed by alcohol *O*-acetyltransferases (Atfs) carrying an alcohol acyltransferase (AATase) domain, primarily Atf1 and Atf2, which together account for approximately half of the total acetate ester synthesized (Fig. 1A) (Verstrepen et al. 2003). While Atf1 is directly involved in volatile ester production, Atf2 is implicated in sterol detoxification via acetylation (Tiwari et al. 2007). Genomic comparisons have shown that only members of *Saccharomyces sensu stricto* contain high-identity orthologs of both *ATF1* and *ATF2* (Van Laere et al. 2008). However, Atf diversity is notably detected in non-*Saccharomyces* yeasts. For example, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, and *Lachancea kluyveri* carry only one low-identity *ATF2*-like ortholog (Gethins et al. 2015). *Hanseniaspora vineae* harbors highly divergent AATase homologs, including an ScAtf2 homolog (26.6% identity) and four Sli1 homologs encoding an *N*-acetyltransferase (Seixas et al. 2023). Despite very low homology to ScAtf homologs, deletion of *HuATF1* significantly reduced acetate ester formation in a diploid *H. uvarum* strain (Badura et al. 2021). *Wickerhamomyces anomalus* possessed functional five WaAtf homologs, which increased isoamyl acetate levels when expressed in *S. cerevisiae* (Kruis et al. 2017). In contrast, *Saccharomycopsis fibuligera* KJJ81, widely used in indigenous food fermentation with rice and traditional Asian alcoholic starters for rice wine, carries 12 *ATF*-like genes in its diploid genome, but only three homologs were validated as functional AATases. (Moon et al. 2021). Similarly, none of the four Atf homologs, except for WsAtf5, showed AATase activity in *Wickerhamomyces subpelliculosus*; however, WsAtf5 was grouped with other yeast Sli1 homologs in the phylogenetic tree and its heterologous expression in *S. cerevisiae* conferred resistance to myriocin, a sphingolipid biosynthesis inhibitor. This indicates that WsAtf5 is a functional homolog of ScSli1, which has *N*-acetyltransferase activity toward myriocin, rather than a conventional AATase involved in flavor production (Momoi et al. 2004, Yoo et al. 2024). These findings suggest the frequent evolutionary loss of AATase function among non-*Saccharomyces* yeast species. This inference is supported by a previ-

Table 1. Volatile flavors synthesized by yeast.

Type	Example	Feature	Major products	References
Acetate ester	Ethyl acetate	Sweet, fruity	Fermented alcoholic beverages, including beer, wine, and sake	Verstrepen et al. (2003), Saerens et al. (2010)
	Butyl acetate	Sweet, banana		
	Isobutyl acetate	Fruity, banana		
	Isoamyl acetate	Banana		
	2-Phenethyl acetate	Rose, honey		
Medium-chain fatty acid (MCFA)-ethyl ester	Ethyl butyrate	Pineapple	Beer and wine	Saerens et al. (2008)
	Ethyl hexanoate	Fruity, green apple		
	Ethyl octanoate	Apple, aniseed, sweet		
	Ethyl decanoate	Floral		
Benzenoid compound	Benzyl alcohol	Mild, sweet, floral	Wine	Martin et al. (2016)
	Benzaldehyde	Almond		
	4-Hydroxybenzyl alcohol	Fruity, sweet, coconut		
	4-Hydroxybenzaldehyde	Woody, vanilla		
Volatile sulfur compound	Dimethyl sulfide (DMS)	Asparagus, corn, molasses, truffle	Cheese, beer, and wine	Landaud et al. (2008)
	Dimethyl disulfide (DMDS)	Garlic	Cheese	
	Hydrogen sulfide (H_2S)	Rotten egg	Cheese, off-flavor in beer and wine	
	Methanethiol (methyl mercaptan, MeSH)	Cooked cabbage, garlic, onion	Cheese, off-flavor in beer and wine	
	Methionol (3-(methylthio)-1-propanol)	Onion, cabbage, cauliflower	Wine, cheese, and soy sauce	Etschmann et al. (2008), Landaud et al. (2008)
	Methional (3-(methylthio)-propionaldehyde)	Mashed potato	Beer and wine	Etschmann et al. (2008)
	Ethanethiol	Onion, rubber		Landaud et al. (2008)
	S-ethyl thioacetate	Fruity, sulfurous, coffee		
	Diethyl disulfide	Garlic, burnt rubber		
	3-Mercaptohexan-1-ol (3MH), 3-Mercaptohexyl acetate (3MHA)	Grapefruit, guava, passion fruit	Beer and wine	Cordente et al. (2019), Michel et al. (2019)
	4-Mercapto-4-methylpentan-2-one (4MMP)	Box tree, blackcurrant	Beer and wine	
	Furfurylthiol	Roasted coffee	Baijiu and wine	Landaud et al. (2008), Zha et al. (2018)
Phenolic compound	4-Vinylguaiacol (4-VG), 4-Ethylguaiacol (4-EG)	Spicy, smoky, clove	Wheat beer and soy sauce	Mukai et al. (2010), Mizuno et al. (2025)
	4-Vinylphenol (4-VP), 4-Ethylphenol (4-EP)	Medicinal, smoky	Off-flavor in wine	Di Toro et al. (2015)
	4-Vinylcatechol (4-VC) 4-Ethylcatechol (4-EC)	Bitter, horsey, barnyard	Roasted coffee, off-flavor in wine	Frank et al. (2007), Milheiro et al. (2019)

ous finding on the absence of any of *ATF1* and *ATF2* genes in some yeast species from the genera *Zygosaccharomyces* and *Torulaspora* identified by *in silico* analysis using the Yeast Gene Order Browser (Gethins et al. 2015). It remains to be investigated how they still produce acetate esters in the absence of canonical *ATF* genes—possibly through alternative pathways or enzymes like Eat1.

MCFA ethyl esters are biosynthesized by the condensation of ethanol and acyl chain derived from fatty acyl-CoA to generate ethyl butyrate (pineapple-like aroma), ethyl hexanoate (aniseed and apple-like aroma), ethyl octanoate (sour apple aroma), and ethyl decanoate (floral aroma) (Fig. 1A) (Saerens et al. 2010). In *S. cerevisiae*, the biosynthesis of MCFA ethyl esters is mediated by two paralogs, Eeb1 and Eht1, which belong to another group of AATases with an α/β hydrolase fold and the Ser-Asp/Glu-His catalytic triad. Eeb1 is more critical in MCFA ester biosynthesis, whereas Eht1 shows a substrate preference for octanoyl-CoA and localizes to lipid droplets, which suggests a role in MCFA detoxification (Knight et al. 2014, Zhu et al. 2019). Most non-*Saccharomyces* species contain only Eht1, with the exception of *K. marxianus*, which retains both Eht1 and Eeb1 (Löbs et al. 2018). Eht1 homologs have been reported in *H. vineae*, *Pichia pastoris*, and *W. subpelliculosus* (Giorello et al. 2019, Chen et al. 2019, Yoo et al. 2024).

Eat1 is a recently identified mitochondrial ethanol acetyltransferase, belonging to the AATase family with an α/β hydrolase fold and the Ser-Asp/Glu-His catalytic triad. It contributes significantly to ethyl acetate production in both *W. anomalus* and *S. cerevisiae* (Fig. 1A) (Kruis et al. 2017, Holt et al. 2018). Eat1 uses short-chain acyl-CoAs, such as acetyl-CoA and propionyl-CoA in the mitochondria, and it facilitates volatile ester formation under suboptimal growth conditions (e.g. iron or oxygen limitation) by converting excess acetyl-CoA or propionyl-CoA to ethyl acetate or ethyl propionate (Kruis et al. 2018b, Kruis et al. 2019). Intriguingly, the α/β hydrolase fold enzymes with AATase activity, including Eat1, Eeb1, and Eht1, exhibited side activities such as esterase,

Table 2. Genome-related information and representative aroma compound pathways in food yeast species.

Species	*Accession number (strain)	Genome size (Mb)	Major aroma compounds (genes)	Aroma-pathways	References
Brettanomyces bruxellensis	GCA_011074885.2 (AWRI2804)	13.2	4-VP, 4-VG (*PAD*), 4-EG, and 4-EP (*VPR*)	HCA decarboxylation, vinylphenol reduction	Granato et al. (2015), Ogata and Saito (2024)
Hanseniaspora guilliermondii	GCA_900119595.1 (UTAD222)	9	Acetate esters (*HgAAT1*, *HgAAT2*, *HgAAT3*, and *HgAAT4*)	Ehrlich	Seixas et al. (2023)
Hanseniaspora uvarum	GCA_050230735.1 (NRRL Y-1614)	9	Ethyl acetate (*EATH*)		Ni et al. (2025)
Hanseniaspora vineae	GCA_000585475.3 (T02/19AF)	11.3	2-Phenylethyl acetate (*ATF2* and *SLI1*), 2-phenylethanol, and benzenoids (*ARO8*, *ARO9*, and *ARO10*)	Ehrlich, shikimate, phenylpyruvate, mandelate	Martin et al. (2016), Giorello et al. (2019)
Kluyveromyces lactis	GCA_000002515.1 (NRRL Y-1140)	10.7	Acetate esters (*KlATF*)	Ehrlich	Van Laere et al. (2008)
Kluyveromyces marxianus	GCA_001417885.1 (DMKU3-1042)	10.9	Acetate esters (*ATF*, *EAT1*, and *IAH1*)	Ehrlich	Gethins et al. (2015), Löbs et al. (2018)
Pichia kluyveri	GCA_030062975.1 (APC 11.10 B)	10.9	Acetate esters (isoamyl acetate and 3-sulfanylhexyl acetate) and thiols	Ehrlich	Vicente et al. (2021)
Saccharomyces cerevisiae	GCA_000146045.2 (S288C)	12.1	Acetate esters (*ATF1* and *ATF2*) and MCFA ethyl esters (*EEB1* and *EHT1*)	Ehrlich	Verstrepen et al. (2003), Saerens et al. (2006)
Saccharomycopsis fibuligera	GCA_001936155.1 (KPH12)	19.6	Acetate esters (*SfATF(A)1–6*, *SfATF(B)1–6*)	Ehrlich	Moon et al. (2021)
Wickerhamiella versatilis	GCA_001600375.1 (JCM 5958)	9.3	4-VG (*FDC1*) and 4-EG (*VRD1*)	HCA decarboxylation, vinylphenol reduction	Hou et al. (2016)
Wickerhamomyces anomalus	GCA_001661255.1 (NRRL Y-366–8)	14.1	Ethyl acetate (*EAT1*) and acetate esters (*ATFs*)	Ehrlich	Kruis et al. (2017)
Wickerhamomyces subpelliculosus	GCA_046457555.1 (CBS 5767)	16.3	Ethyl acetate (*EAT1*) and acetate esters (*ATF1*, *ATF2*, *ATF3*, and *ATF4*)	Ehrlich	Yoo et al. (2024)

*Information on genomes was obtained from haploid yeast strain.

thioesterase, and alcoholysis. Particularly, Eat1 exhibited robust alcoholysis activity, which was ∼450 times higher than its AATase activity (Patinios et al. 2020). Notably, acetate ester production in several non-*Saccharomyces* species appears to be mediated by the α/β hydrolase fold enzyme family, such as Eat1 and EatH (an Eat1 homolog of *H. uvarum*), rather than by Atf homologs. For example, CRISPR-based gene knockout and truncation studies showed that Eat1 is essential for ethyl acetate, isoamyl acetate, and phenethyl acetate formation in *K. marxianus* (Löbs et al. 2018). Similarly, the EatH enzyme in *H. uvarum* showed a broad alcohol substrate spectrum (Ni et al. 2025).

Besides AATase activities, ester levels are additionally regulated by other underlying mechanisms such as reverse esterase activity and hemiacetal dehydrogenation (Park et al. 2009). The ester yield is primarily determined by substrate availability and enzyme activity in ester synthesis and hydrolysis (Fukuda et al. 1998, Verstrepen et al. 2003). *IAH1* encodes an isoamyl acetate-hydrolyzing esterase, and its deletion increased ester accumulation in *S. cerevisiae* (Fukuda et al. 1998). Similarly, *TIP1* is another esterase gene, and its deletion enhanced ethyl ester levels (Dank et al. 2018). However, ester profiles reverted to the wild-type in the double mutant (*iah1*Δ*tip1*Δ), which suggests a compensatory regulation in aroma formation (Dank et al. 2018). Comparative genomics of *Cyberlindnera fabianii* and *Pichia kudriavzevii*—lacking either Atf1 or both Atf1 and Atf2, respectively, but both possessing Eat1 and Iah1—confirmed that acetate ester levels were primarily regulated by hydrolase activity (van Rijswijck et al. 2019). Moreover, CRISPRi-mediated repression of *IAH1* in *K. marxianus* reduced ethyl acetate but increased ethyl butyrate and alcohol levels, implying a role as ester synthase or activation of alternative enzymes (Munoz-Miranda et al. 2024). Overall, these findings highlight the remarkable genetic diversity in ester biosynthesis across yeast species. Thus, both the evolutionary divergence of AATase families and the compensatory mechanisms of alternative pathways, such as Eat1- and esterase-mediated routes, collectively influence the aroma of fermented products.

Genome-driven reconstruction of the benzenoid biosynthesis pathway

Volatile benzenoids are particularly important in cosmetic, pharmaceutical, fragrance, and food industries as they render floral and fruity aromas to final products. For example, benzyl alcohol adds mild, sweet, and floral notes, whereas benzalde-

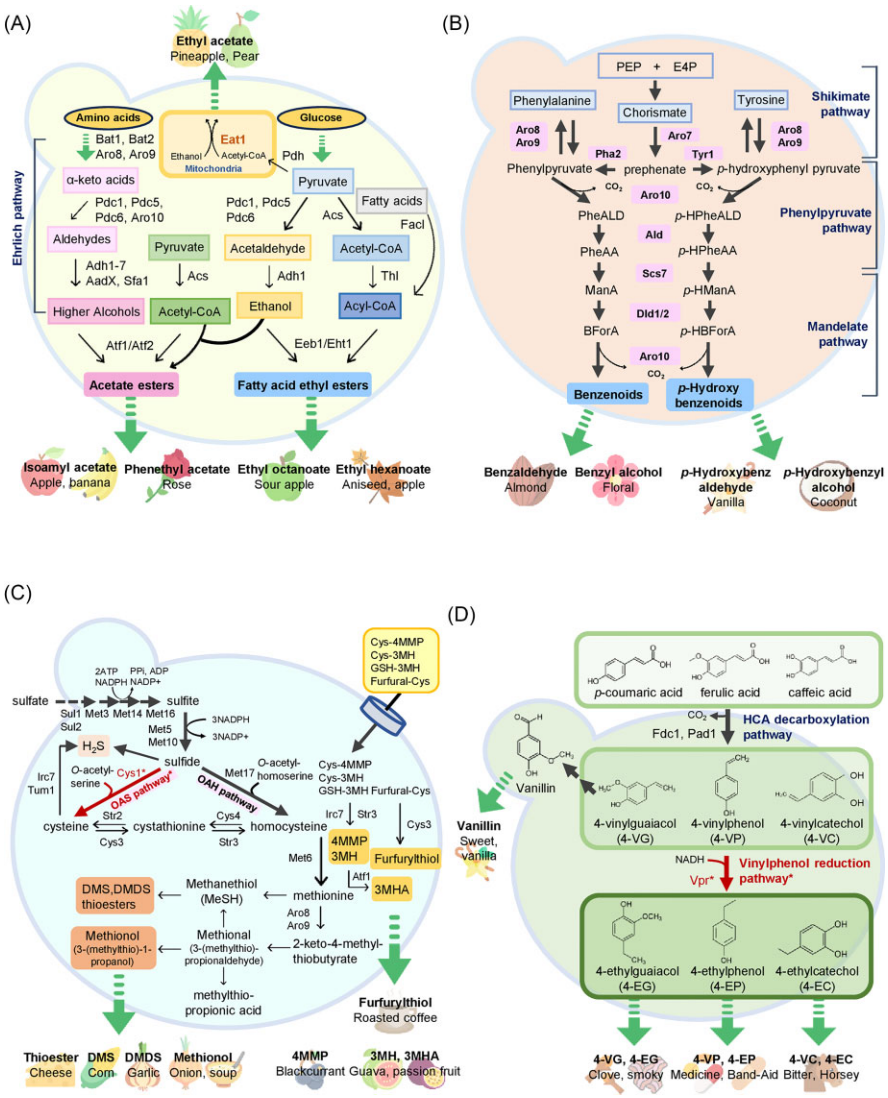

Figure 1. Genome-informed biosynthetic pathways of volatile flavor compounds in yeasts. (A) Ester biosynthesis in *S. cerevisiae*: Acetate esters are synthesized by Atf1/Atf2 enzymes through the condensation of acetyl-CoA and higher alcohols, whereas MCFA ethyl esters are synthesized by Eeb1/Eht1 enzymes from ethanol and acyl-CoAs. The synthesis of ethyl acetate is mainly mediated by Eat1 in the mitochondria. AadX (aryl alcohol dehydrogenase: Aad3, 4, 6, 10, 14, 15, and 16), Acs (acetyl-CoA synthetase), Adh1-7 (alcohol dehydrogenases), Aro8, Aro9 (aromatic aminotransferase), Aro10 (phenylpyruvate decarboxylase), Atf1, Atf2 (alcohol *O*-acetyltransferase), Bat1, Bat2 (branched-chain amino acid aminotransferase), Eeb1 (acyl-coenzyme A:ethanol *O*-acyltransferase), Eht1 (octanoyl-CoA:ethanol acyltransferase), Facl (fatty acid CoA-ligase), Pdc1, Pdc5, Pdc6 (pyruvate decarboxylase), Pdh (pyruvate dehydrogenase), Sfa1 (bifunctional alcohol dehydrogenase and formaldehyde dehydrogenase), and Thl (thiolase). (B) Biosynthetic pathway of benzenoid compounds in *S. cerevisiae* and *H. vineae*: Chorismate is synthesized from phosphoenolpyruvic acid (PEP) and erythrose-4-phosphate (E4P) via the shikimate pathway. Phenylalanine and tyrosine are subsequently derived from chorismate through specific branches. The intermediates, phenylpyruvate and *p*-hydroxyphenyl pyruvate, from phenylalanine and tyrosine, respectively, or from prephenate, are further transformed to benzaldehyde and *p*-hydroxybenzaldehyde, respectively, by the action of Aro10, Ald (aldehyde dehydrogenase), Scs7 (sphingolipid α-hydroxylase), and Dld1/2 (D-lactate dehydrogenase) via the phenylpyruvate/mandelate pathways. Aro7 (chorismate mutase), Pha2 (prephenate dehydratase), Tyr1 (prephenate dehydrogenase involved in tyrosine biosynthesis), PheALD (phenylacetaldehyde), PheAA (phenylacetic acid), ManA (mandelic acid), BForA (benzoylformic acid), *p*-HPheALD (*p*-hydroxyphenylacetaldehyde), *p*-HPheAA (*p*-hydroxyphenylacetic acid), *p*-HManA (*p*-hydroxymandelic acid), and *p*-HBForA (*p*-hydroxybenzoylformic acid). (C) Sulfate assimilation and VSC formation in *S. cerevisiae*: Sulfate is reduced to sulfide, which is incorporated into the carbon chains to form homocysteine via *O*-acetylhomoserine (OAH) pathway. *O*-acetylserine (OAS) pathway is absent in *S. cerevisiae*, but present in several yeast species including *Ogataea parapolymorpha* and *Schizosaccharomyces pombe*. Methionine is further metabolized to produce methanethiol (MeSH), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), thioesters, methional (3-(methylthio)-propionaldehyde), methionol (3-(methylthio)-1-propanol), and other VSCs. Aromatic thiols, such as 3-mercaptohexan-1-ol (3MH), 4-mercapto-4-methylpentan-2-one (4MMP), and furfurylthiol are released from their odorless precursors conjugated with cysteine (Cys) or glutathione (GSH) by the carbon–sulfur lyases, Irc7 (cysteine desulphydrase), Str3 (cystathionine β-lyase), or Cys3 (cystathionine γ-lyase). 3MH can be further converted to 3-mercaptohexyl acetate (3MHA) by Atf1. Hydrogen sulfide (H_2S) is formed from cysteine by Irc7 or Tum1 (rhodanese domain sulfur transferase). Sul1, Sul2 (sulfate permease), Met3 (ATP sulfurylase), Met14 (adenylylsulfate kinase), Met16 (3'-phosphoadenylsulfate reductase), Met5, Met10 (sulfite reductase), Met17 (*O*-acetyl homoserine-*O*-acetyl serine sulfhydrylase), Met6 (methionine synthase), Cys1 (*O*-acetylserine sulfhydrylase), Cys4 (cystathionine β-synthase), and Str2 (cystathionine γ-synthase). (D) Biosynthesis of phenolic compounds in yeast: Hydroxycinnamic acids (HCAs) are decarboxylated by ferulic acid decarboxylase 1 (Fdc1) with a cofactor produced by phenylacrylic acid decarboxylase 1 (Pad1) to yield 4-vinyl derivatives in *S. cerevisiae*. Only in a few yeast species, such as the yeasts of the genera *Brettanomyces* and *Dekkera*, *C. fermentati* and *W. versatilis*, but not in *S. cerevisiae*, these compounds are further reduced by vinylphenol reductase (Vpr) to form their ethyl counterparts that contribute spicy, smoky, and medicinal aroma notes. The arrows, enzymes, and pathways shown in red (and also indicated by an asterisk (*)) indicate their absence in *S. cerevisiae*. Flavor icons and arrows are the resources from Flaticon.com.

hyde contributes almond and dry fruit flavors in wine varieties (Scognamiglio et al. 2012, Martin et al. 2016). Additionally, *p*-hydroxybenzyl compounds, such as *p*-hydroxybenzyl alcohol and *p*-hydroxybenzaldehyde provide fruity, sweet, coconut, woody, or vanilla flavors (Martin et al. 2016). Contrary to the conventional perception that benzenoid compounds originate from grapes and not from fermentative processes, Martin et al. (2016) have suggested the *de novo* formation of these compounds from aromatic amino acids and sugars in yeast based on the observation of higher levels of benzyl alcohol in wine than in grape juice. Comparison of the genomic information of *H. vineae* isolated from vineyards with those of *S. cerevisiae* and plants suggested a putative biosynthetic pathway for benzenoids in yeast (Martin et al. 2016). Benzyl alcohol, benzaldehyde, *p*-hydroxybenzaldehyde, and *p*-hydroxybenzyl alcohol are synthesized through the chorismate pathway from sugars and the aromatic amino acids phenylalanine and tyrosine (Fig. 1B). The intermediates phenylpyruvic acid and *p*-hydroxyphenyl pyruvic acid, generated from phenylalanine and tyrosine, respectively, or from prephenic acid, are transformed into mandelic acid (ManA) and *p*-hydroxymandelic acid (*p*-HManA), respectively, by the action of Aro10 (phenylpyruvate decarboxylase), Ald (aldehyde dehydrogenase), and Scs7 (sphingolipid alpha-hydroxylase) in the phenylpyruvate pathway. Then, benzaldehyde and *p*-hydroxybenzaldehyde are synthesized via the action of Dld1/2 (D-lactate dehydrogenase) and Aro10 in the mandelate pathway and are subsequently converted to benzyl alcohol, benzoic acid, *p*-hydroxybenzyl alcohol, and *p*-hydroxybenzoic acid (Fig. 1B) (Valera et al. 2020a). Particularly, *p*-hydroxybenzoic acid acts as a quinone ring precursor for the synthesis of coenzyme Q, which functions in the electron transport chains of the mitochondria and the plasma membrane and is an important antioxidant in both human and yeast cells (Valera et al. 2020a, Clarke 2000).

The Aro10 protein of *S. cerevisiae*, mainly involved in decarboxylating phenylpyruvate into phenylacetaldehyde in the Ehrlich pathway (Fig. 1A; Vuralhan et al. 2003), exhibits broad substrate specificity (Kneen et al. 2011). Besides its initial decarboxylation of (*p*-hydroxy)phenylpyruvate into (*p*-hydroxy)phenylacetaldehyde (PheALD and *p*-HPheALD) in the benzenoid biosynthesis, Aro10 is also involved in the later decarboxylation step, in which (*p*-hydroxy)benzoylformate (BForA and *p*-HBForA) is converted to (*p*-hydroxy)benzaldehyde, playing an additionally critical role for benzyl alcohol production (Valera et al. 2020b). *H. vineae* strains produced 20–200 times higher quantities of benzyl alcohol than *S. cerevisiae* strains in the synthetic grape fermentation medium (Martin et al. 2016). Inferred from analyses of sequence and structure along with expression patterns, two *ARO10* homologous genes, *HvARO10A* and *HvARO10B*, were identified to possess benzoylformate decarboxylase activity in the whole genomes of 11 *H. vineae* strains, which may explain the high benzyl alcohol production by this yeast species compared with that of *S. cerevisiae* (Valera et al. 2020a,b). Similarly, *W. subpelliculosus* generated more benzaldehyde than *S. cerevisiae*, and three *ARO10* copies were found in its genome, although further detailed investigations are needed (Yoo et al. 2024).

Genome-guided VSC biosynthesis pathways

VSCs belong to a class of the most notable aroma-active molecules in wine and are regarded as a "double-edged sword" because they exert both positive and negative impacts on the perception of wine quality (Swiegers and Pretorius 2007). Furthermore, they contribute to the aromatic complexity and uniqueness of fermented foods or beverages, such as cheese or wine. Undesirable VSCs, including hydrogen sulfide (H_2S), methanethiol (MeSH), and methylthioesters, are associated with distinct off-flavors characterized as rotten egg, cooked cabbage, and onion-chive, respectively, even at low concentrations. VSCs produced by yeast are derived from sulfate assimilation pathway and sulfur-containing amino acid biosynthetic pathways (Fig. 1C). Extracellular inorganic sulfate imported into yeast cells by sulfate permeases encoded by *SUL1* and *SUL2* is activated and reduced to sulfide by the action of the several *MET* genes including the *MET3*, *MET14*, *MET16*, *MET5*, and *MET10* genes using two ATP molecules and four NADPH molecules (Thomas and Surdin-Kerjan 1997). In *S. cerevisiae*, sulfide is incorporated into the four-carbon chain *O*-acetylhomoserine by *O*-acetyl homoserine sulfhydrylase, encoded by *MET17* to generate homocysteine (*O*-acetylhomoserine pathway, OAH pathway), which is converted to methionine and then cysteine via a transsulfuration pathway. In contrast, in several yeast species such as *Ogataea parapolymorpha*, sulfide is condensed with the three-carbon chain *O*-acetylserine by *O*-acetylserine sulfhydrylase, encoded by *CYS1*, to directly generate cysteine (*O*-acetylserine pathway, OAS pathway) (Thomas and Surdin-Kerjan 1997, Sohn et al. 2014). Notably, genomic and transcriptomic analyses showed that the open reading frames (ORFs) required for sulfate assimilation were absent in *S. fibuligera* (Choo et al. 2016), indicating its unique sulfur pathway. *Schizosaccharomyces pombe*, *S. fibuligera*, and several filamentous fungi such as *Aspergillus nidulans* isolated in fermented foods house both the OAH and OAS pathways (Marzluf 1997, Fujita and Takegawa 2004, Choo et al. 2016). Despite the absence of sulfate assimilation pathway, numerous genes for extracellular hydrolytic enzymes, such as acidic proteases, in the *S. fibuligera* genome might help degrade proteins from the environment to obtain amino acids as sulfur sources, during food fermentation on various grains. Consistent with this, the expression patterns of these extracellular hydrolytic enzymes generally appeared to be induced under sulfur-limited conditions (Choo et al. 2016).

Excess sulfide causes H_2S overproduction, which diffuses from the cells into the fermentation medium under conditions of nitrogen starvation or abundance of sulfur sources (Jiranek et al. 1995). Methionine is catabolized via transamination or demethylation reactions to MeSH, which is a key precursor for numerous VSCs, followed by further oxidation to generate dimethyl sulfide, dimethyl disulfide, and thioesters that impart boiled cabbage, sulfurous, garlic, and cheesy aromas (Landaud et al. 2008, Zhang et al. 2016, Dzialo et al. 2017). In *S. cerevisiae*, 3-(methylthio)-1-propanol, also known as methionol, is derived from methionine via the Ehrlich pathway. L-methionine is transformed into 2-keto-4-methyl-thiobutyrate by transaminases and converted to methional (3-(methylthio)-propionaldehyde) and then methionol (Etschmann et al. 2008), a VSC produced during fermentation by yeasts. It imparts off-flavors reminiscent of cauliflower and boiled potato to wine and distinctive aromas to many food products such as cheese, potato, and soy-based products (Lopez Del Castillo-Lozano et al. 2007, Etschmann et al. 2008, Quek et al. 2011, Deed et al. 2019). A previous study on deletion mutants showed that the aromatic aminotransferases Aro8 and Aro9 function differently between the lab and wine strains for methionol production (Deed et al. 2019). The *aro8*Δ deletion resulted in a 44% decrease in the lab strain and 92% reduction in the wine strain, representing the inability of the wine strain to catabolise methionine to methionol in the absence of Aro8. A functional Aro9, or even func-

tional branched-chain amino acid aminotransferases Bat1/Bat2, is not sufficient to compensate for the role of Aro8 in the wine strain. The reduction in methionol for the BY4743 *aro8* deletant could be ascribed to the higher aminotransferase activity of Aro8 compared to Aro9. Deletion of *ARO9* also led to an unexpected 46% increase in methionol in the wine strain, but no effect in the lab strain, suggesting the possibility of Aro9 as a repressor of methionine catabolism in the wine strain or Aro8 as a limiting step for methionol production. In conclusion, the results suggest that the Ehrlich pathway, which includes Aro8 and Aro9, functions differently between the *S. cerevisiae* lab and wine strains for methionol generation (Deed et al. 2019).

In contrast, representative positive VSCs include polyfunctional thiols, such as 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA), which are associated with tropical, passionfruit, grapefruit, guava, and box tree aromas especially detected in beer and wine (Cordente et al. 2019, Michel et al. 2019). 4MMP and 3MH exist in grapes or hops as nonvolatile precursor forms, conjugated to cysteine or glutathione, Cys-4MMP, Cys-3MH, and GSH-3MH (Fedrizzi et al. 2009, Capone et al. 2010, Cordente et al. 2019, Michel et al. 2019). Furfurylthiol is another important aromatic thiol with a scent reminiscent of roasted coffee, which is formed from its precursor furfural–cysteine conjugates. The odorless precursors are imported into cells via amino acid transporters and cleaved to release the corresponding free aromatic thiols by carbon–sulfur β-lyase activity of several yeast species including *S. cerevisiae*, *S. pastorianus*, and *Torulaspora delbrueckii* (Fig. 1C) (Subileau et al. 2008, Pretorius et al. 2012, Cordente et al. 2019). *S. cerevisiae IRC7* encodes cysteine desulphydrase, which is responsible for releasing free 3MH and 4MMP from their cysteinylated precursors, whereas *STR3* encodes cystathionine β-lyase, which shows modest side activity for these aroma compounds (Fig. 1C) (Holt et al. 2012, Harsch and Gardner 2013). Carbon–sulfur β-lyases encoded by *CYS3* and *STR3* cleave furfural–cysteine conjugates to release furfurylthiol, pyruvate, and ammonia (Zha et al. 2018, Zhang et al. 2023). Furthermore, Atf1, an alcohol *O*-acetyltransferase encoded by *ATF1*, converts 3MH to its ester form 3MHA, which exhibits tropical fruit notes (Pretorius et al. 2012).

Additionally, cysteine is a factor that induces H_2S production by the action of *IRC7*- or *TUM1*-encoded enzymes to generate ammonium, pyruvate, and H_2S (Jiranek et al. 1995, Huang et al. 2017). Recently, QTL mapping has shown that four *S. cerevisiae* genes that are not associated with sulfur metabolism affect H_2S formation under winemaking conditions (De Guidi et al. 2024). They are *ZWF1*, encoding a cytoplasmic glucose-6-phosphate dehydrogenase involved in pentose phosphate pathway in relation to NADPH availability; *ZRT2*, which encodes a high affinity zinc transporter; *SRN2*, which encodes an endosomal sorting complex component required for the transport machinery that participates in the degradation of amino acid permeases; and YLR125W, which is an uncharacterized gene. This shows that H_2S production is closely linked to cell physiology through NADPH and Zn^{2+} availability, status of the protein degradation machinery, and an unknown mechanism related to YLR125W possibly through interactions with regulators of the sulfur pathway (De Guidi et al. 2024). Furthermore, a previous study using an isotope labeling and the *S. cerevisiae met17*Δ strain demonstrated that VSCs, such as ethanethiol, S-ethyl thioacetate, and diethyl disulfide that present cooked onion or vegetable aroma may be directly synthesized from H_2S perhaps by a reaction of H_2S with acetaldehyde or ethanol, via an uncharacterized mechanism that adds an acetyl group to ethanethiol, and via dimerization of ethanethiol by a chemical oxidation, respectively (Kinzurik et al. 2016).

Comparative genomic analysis of phenolic compound biosynthetic pathways

Volatile phenols can contribute either desirable aromas or off-flavors, depending on the type of food. They originate from hydroxycinnamic acids (HCAs), which are phenolic compounds derived from the secondary metabolism of plants and commonly found in fruits, grains, bran, and herbs. These compounds include caffeic acid, chlorogenic acid, *p*-coumaric acid, and ferulic acid (FA), which are crucial for plant immunity and exhibit antimicrobial activity (Liu et al. 2022).

To defend against the negative effect of HCAs, many bacteria and fungi have evolved pathways to convert HCAs to less toxic molecules with two sequential enzymatic reactions (Fig. 1D). First, HCAs are decarboxylated into the corresponding vinyl derivative. Typically, 4-vinylphenol (4-VP), 4-vinylcatechol (4-VC), and 4-vinylguaiacol (4-VG) are produced from *p*-coumaric acid, caffeic acid, and FA, respectively. These vinyl derivatives are further reduced to their corresponding ethyl derivatives 4-ethylphenol (4-EP), 4-ethylcatechol (4-EC), and 4-ethylguaiacol (4-EG), respectively (Nunes de Lima et al. 2021, Lopez de Felipe 2023). Both 4-VG and 4-EG are linked to objectively pleasant clove-like, smoky, or spicy flavors, whereas 4-VP and 4-EP are often described as medicinal and reminiscent of "Band-Aid" (Dzialo et al. 2017). These volatile phenolic compounds contribute substantially to fermentation aromas, but they are perceived differently based on the product. For example, although 4-VG is regarded as an off-flavor in standard beers, it is an essential characteristic aroma of soy sauce, miso, and certain types of beer, such as Belgian wheat ales and German Hefeweizen beers at levels below the threshold value (Suezawa and Suzuki 2007, Mukai et al. 2010, Steensels and Verstrepen 2014).

In *S. cerevisiae*, decarboxylation of HCAs requires two enzymes, described as phenylacrylic acid decarboxylase (Pad1) encoded by *PAD1* and ferulic acid decarboxylase (Fdc) encoded by *FDC1* (Fig. 1D). Pad1 acts as a prenyltransferase that synthesizes the prenylated flavin mononucleotide (FMN) cofactor, which is essential for Fdc1's decarboxylase activity (Lin et al. 2015, Payne et al. 2015). A recent study based on comparative genomics of *S. cerevisiae* industrial strains reported that the structural features of *FDC1* and *PAD1* are associated with 4-VG production capacity (Son et al. 2023). Three main types of variation were observed in the genomic structures of *FDC1* and *PAD1* among 66 *S. cerevisiae* strains from different geographic and environmental origins: (i) mutations in one or both genes, mostly early stop codons, but also deletions and amino acid substitutions; (ii) differences in the intergenic distance between the two genes; and (iii) variation in gene orientation, with the genes arranged in opposite or the same transcriptional directions depending on the strain. Although the genomic distance between the two genes was 463 bp in most cases, the CEN.PK2 lab strain genome showed a 643 bp distance. Additionally, CEN.PK2-1C showed a mutation of the early stop codon in *PAD1*, inverted *PAD1* orientation, and partial deletion of *FDC1* (Richard et al. 2015, Son et al. 2023), which resulted in the loss of 4-VG production activity in CEN.PK2-1C. Similarly, the rice wine-brewing KSD-YC industrial strain did not show 4-VG production activity because of a premature stop codon caused by a nonsense mutation at position 54 in the Fdc1 protein. These findings support a strong correlation between the variations in the *PAD1* and *FDC1* genes and the FA decarboxylation capacity of yeast strains,

indicating the selection of domesticated brewing yeasts that are unable to produce this specific off-flavor (Son et al. 2023).

The halophilic yeast *Debaryomyces hansenii* isolates, *D. hansenii* KD2 and *D. hansenii* C11 from Korean soy sauce, showed strong bioconversion activity from FA to 4-VG under high-salt conditions (15% NaCl), indicating that this salt tolerance may contribute to the distinctive flavor profile of *D. hansenii* in soy sauce fermentation (Jeong et al. 2022). Interestingly, distinctive 4-VG production activity was observed in the two *D. hansenii* strains: KD2 showing high salt-dependent 4-VG biosynthetic activity, whereas C11 displayed constitutive 4-VG conversion activity. However, molecular mechanisms underlying the different salt-dependent 4-VG biosynthetic activity have not yet been reported. Additionally, *D. hansenii* can generate commercially valuable vanillin after spontaneous decarboxylation of 4-VG (Mathew et al. 2007).

Further conversion of HCA vinyl derivatives into ethyl derivatives, such as 4-EG, is catalysed by vinylphenol reductase (Vpr) (Fig. 1D). However, only a few yeast species, such as the yeasts of the genera *Brettanomyces* and *Dekkera*, *Candida fermentati*, and *Wickerhamiella versatilis*, possess Vpr (Chatonnet et al. 1992, Suárez et al. 2007, Suezawa and Suzuki 2007). *Brettanomyces bruxellensis* is a red wine spoilage yeast associated with phenolic aromas described as medicinal, horsy, and paint, which diminish the sensory qualities of wine (Di Toro et al. 2015). A comparative transcriptome and genome-wide analysis of *B. bruxellensis* LAMAP2480 during *p*-coumaric acid stress showed *PAD1* overexpression (Godoy et al. 2016). However, a 4-VG formation mechanism that differs from the one in *S. cerevisiae* has been recently suggested in *B. bruxellensis* (Ogata and Saito 2024). *Brettanomyces bruxellensis* phenolic acid decarboxylase catalyses the conversion of FA to 4-VG without requiring prenylated FMN as a cofactor, in contrast to *S. cerevisiae* Pad1 (Ogata and Saito 2024). Additionally, heterologous expression of *B. bruxellensis* Vpr in *S. cerevisiae* triggered the reduction of 4-VG, producing 4-EG (Romano et al. 2017). Interestingly, purified *B. bruxellensis* Vpr exhibited significant superoxide dismutase (Sod) activity responsible for detoxification of reactive oxygen species (Granato et al. 2015). This suggests that *B. bruxellensis* Vpr exhibits dual functionality as a Sod, indicating the survival strategy of *B. bruxellensis* in wine, in which Sod activity may counteract the oxidative stress and Vpr activity could contribute to balancing the $NAD^+/NADP^+$ requirements necessary to sustain active metabolism (Romano et al. 2017).

Wickerhamiella versatilis also produces high levels of 4-EG, which contributes to the richness of the characteristic soy sauce aroma, and the presence of a *W. versatilis* Vpr homolog was indicated (Hou et al. 2016, Mizuno et al. 2025). However, the sequence and function of the *W. versatilis* Vpr homolog remains unclear.

2. Multi-omics analyses of flavor production by yeast during food fermentation

In food biotechnology, the "flavor phenotype" results from the combined physiological activities of each member of yeast starter cultures and is a key factor that determines the sensory features of food (Cordente et al. 2012). Recent advances in multi-omics technologies, comprising genomics, transcriptomics, proteomics, and metabolomics, have deepened our understanding of the molecular mechanisms underlying flavor compound biosynthesis in various yeast species. Integration of these diverse datasets provides a holistic view of the biological pathways involved in flavor formation, enabling targeted strain improvement and product innovation (Table 3). For example, the transcriptome analysis of *B. bruxellensis* grown in the presence and absence of *p*-coumaric acid with the high-quality genome information revealed that the entrance of *p*-coumaric acid generates a stress condition to the cell, resulting in induced expression of proton pumps and efflux of toxic compounds along with upregulation of the *PAD1* gene, which facilitates decarboxylation of *p*-coumaric acid (Godoy et al. 2016). The comparative study that analyzed the genomic, transcriptomic, and metabolomic profiles of the wine yeast *H. vineae* with those of *S. cerevisiae* identified several changes in the dosage of key genes involved in flavor production including higher alcohols and esters (Giorello et al. 2019). Moreover, the integrated genomic, transcriptomic, and metabolomic analyses of *H. vineae* in combination with the functional analysis in the heterologous host *S. cerevisiae* have led to the identification of a novel biosynthesis pathway for benzenoid compounds in yeast. Yeast exploits the phenylpyruvate/mandelate pathways to synthesize benzenoids, instead of the phenylalanine ammonia-lyase and tyrosine ammonia-lyase pathways, which are found in plants and some filamentous fungi (Valera et al. 2020a, Martin et al. 2016).

Flavor perception is a complex system, not a simple sum of individual compounds, determined by physicochemical binding with the food matrix, synergistic and antagonistic effects among compounds, and the dynamic environment of the fermentation process (van Wyk 2024, Mao et al. 2025). Compared to single-species fermentation, yeast cofermentation received attention recently as a versatile strategy that greatly improves flavor complexity, organoleptic quality, and health-associated traits of fermented foods and beverages. Whereas *S. cerevisiae* has long been the dominant species because of its robust fermentative performance, it often produces limited aroma diversity. In contrast, various non-*Saccharomyces* yeast species are more efficient at producing flavor compounds, such as esters and higher alcohols but have a relatively low alcohol production capacity (Jolly et al. 2014, Ciani and Comitini 2015). A synergistic effect can occur when the metabolic activity of one yeast species modulates the metabolic pathways of another, thereby promoting the production of flavor compounds (Vilela 2020). Thus, cofermentation emerges as a powerful strategy for maximizing flavor complexity through the metabolic interactions between yeast species. Furthermore, cofermentation can increase product yields with improved flavor profiles by combining the abilities of different yeast species to break down and ferment a wider range of components. For example, coculture of *S. cerevisiae* with *S. fibuligera*, an ascomycete with potent amylolytic activity, demonstrates the cooperative interaction between *S. fibuligera* and *S. cerevisiae* in alcoholic fermentation (Saikia and Saikia 2024). *S. fibuligera* provides the ability to efficiently convert starch into diverse sugars and amino acids, which are metabolized to alcohol, mainly by *S. cerevisiae* and to various flavor compounds by both yeast species, enhancing the sensory attributes of beverages. Recently, a synergistic enhancement of FA metabolism through the 4-VG high-yielding *Starmerella etchellsii* mutant strain, combined with the introduction of *W. versatilis* and the attenuation of *D. hansenii* not only amplifies smoky flavor, but also redirects metabolic flux to promote caramel-like, creamy, and fruity aroma compounds, resulting in a comprehensive optimization of flavor quality of soy sauce (Wang et al. 2025).

The increasing availability of genomic resources and omics tools has enabled genome-wide investigations of gene–phenotype relationships during cofermentation. (Table 3). For instance, comparative transcriptomic analyses of *S. cerevisiae* in monoculture and in coculture with *H. guilliermondii* revealed differential ex-

Table 3. Examples of multi-omics analysis of flavor production in fermentations by single, coculture, and mixed culture of yeast.

Fermentation	Yeast species	Omics technology	Target flavors (culture condition)	Analysed key genes or targets	References
Single culture	*B. bruxellensis*	Genomics, transcriptomics	4-Vinylphenols (synthetic dextrose minimal medium containing *p*-coumaric acid)	*PAD1*	Godoy et al. (2016)
	H. vineae	Genomics, transcriptomics, metabolomics	2-Phenethyl acetate, phenyl propanoids (2-phenylethyl and benzyl alcohols) (Chemically defined grape (CDG) fermentation medium)	*ARO8*, *ARO9*, *ARO10*, *ATF2*, *SLI1*	Giorello et al. (2019)
	H. vineae	Genomics, transcriptomics, metabolomics	Benzenoid compounds (CDG fermentation medium)	*ARO10*, *ALD*, *SCS7*, *DLD1/2*	Martin et al. (2016), Valera et al. (2020a)
Coculture	*H. guilliermondii* + *S. cerevisiae*	Comparative transcriptomics	Higher alcohols, acetate esters, ethyl esters, H_2S (natural grape-juice)	Differentially expressed genes (DEGs) between single and coculture	Barbosa et al. (2015)
	S. pombe + *S. cerevisiae*	Transcriptomics, metabolomics	Esters, alcohols, terpenes, organic acids, polyphenols, free amino acids (apple juice containing sodium pyrosulfite)	DEGs and metabolite profiles between single and coculture	Yu et al. (2022)
	Metschnikowia koreensis + *S. cerevisiae*	Metabolomics	Enhanced aroma complexity: organic acids, free amino acids, VOCs (sterile apple juice)	Quality enhancement of cider and metabolite profiles between single and coculture	Wu et al. (2025)
Mixed culture	Yeast communities associated with grape musts	Meta-genomics, meta-transcriptomics, metabolomics	Alcohols, esters, acids (grape must/synthetic grape must)	DEGs and metabolite profiles by community composition	de Celis et al. (2024)

pression of genes associated with the biosynthesis of higher alcohols, acetate esters, acetaldehyde, ethanol, acetic acid, and H_2S. Additionally, these results potentially explain the differences observed in the aroma profiles of wines by elucidating yeast dynamics during wine fermentation through the genome-wide study of yeast–yeast interactions (Barbosa et al. 2015). *S. pombe* is a yeast with potential in fruit winemaking because of its strong fermentation performance and deacidification capacity. Subsequently, the metabolomic and transcriptomic data between *S. cerevisiae* single culture and coculture with *S. pombe* during apple cider fermentation were analysed (Yu et al. 2022). The transcriptome of the cider pellets showed an obvious association between aroma- and taste-compound formation and differential gene expression networks during the coculture of the two yeasts with increased fruity and flower aroma, decreased sourness, and enhanced umami taste, indicating that the presence of one influences the metabolic pathways of the other (Yu et al. 2022).

A metabolomic study of cider fermentation showed that the acid protease and esterase production capability of *Metschnikowia* spp. increased the levels of esters (e.g. ethyl octanoate and ethyl decenoate) and higher alcohols (e.g. 1-pentanol and phenethyl alcohol), which supports their selection as flavor-enhancing cofermentation partners of *S. cerevisiae* (Wu et al. 2025).

Furthermore, a recent multi-omics study showed that the wine yeast community composition is primarily influenced by environmental factors (i.e. biogeography) rather than anthropic factors (i.e. viticultural practices) (de Celis et al. 2024). By combining transcriptomic and metabolomic analyses, researchers have linked community composition to functional output in fermentation ecosystems. The transcriptomic and metabolite profiles of *Hanseniaspora*-dominated wine samples showed that they were characterized by higher residual sugars, acetic acid, and acetate ester production, whereas *Lachancea*-dominated samples correlated with L-lactic and succinic acid production and fusel alcohols. These findings provide essential insights into identifying a specific set of orthologs that underlie the unique contributions of various yeast species to wine flavor and aroma. Importantly, these findings present two alternative exclusive approaches for crafting customizable wines: precisely controlling native wine yeast communities or carefully designing synthetic microbial consortia, leading to complex and desirable flavors in various fermented foods and beverages such as wine, beer, cider, bread, and soy sauce (de Celis et al. 2024).

Future directions: omics analysis-based synthetic biology of flavor yeasts in industrial applications

As discussed in this review, emerging multi-omics approaches have highlighted the intricate metabolic interplay in yeast cocultures and microbial populations, emphasizing their largely un-

tapped potential for fine-tuning flavor complexity. Future studies should prioritize the integration of genomic, transcriptomic, and metabolomic data with predictive fermentation models to enable the rational design of functionally synergistic microbial consortia. Furthermore, multi-omics data-based genome-scale metabolic models will continue to deepen our understanding of yeast flavor biosynthetic metabolism and provide strong platforms for synthetic biology as the next frontier in flavor research. This discipline offers powerful tools for the rational design and engineering of yeast strains with customized aroma production profiles that are tailored to specific food and beverage matrices. Implementation of synthetic biology in flavor-associated yeasts has already resulted in some practical applications. Engineered *S. cerevisiae* strains now synthesize raspberry ketone, β-ionone, and plant terpenoids by integrating plant genes, transcriptional regulators, and modular biosynthetic elements (Chen et al. 2023). Advanced synthetic biology strategies have enabled the construction of synthetic microbial consortia by engineering metabolic pathways combined with modulating regulatory gene networks, which together enhance aroma production and reduce metabolic burden. For example, Peng et al. (2023) have shown that synthetic cocultures of engineered *S. cerevisiae* strains with modifications in different metabolic modules significantly enhance raspberry ketone biosynthesis by dividing the labor among strains and fine-tuning gene expression modules (Peng et al. 2023). These approaches highlight the usefulness of the compartmentalization of metabolic functions among yeast populations to improve yields and stability. Furthermore, the idea of a "synthetic metagenome" encapsulated in a single *S. cerevisiae* chassis represents a visionary leap. Belda et al. (2021) have proposed the construction of synthetic chromosomes encoding the metabolic capacities of entire microbial communities—especially those of wine fermentation consortia—in a single yeast cell (Belda et al. 2021), facilitating novel approaches to investigate flavor synthesis mechanisms at an unprecedented resolution.

Overall, omics-based synthetic biology offers powerful tools to advance yeast flavor research by moving beyond natural biodiversity toward controlled fermentation systems that integrate genomic insights with industrial applications. These approaches can uncover the genetic and ecological bases of aroma formation and enable the rational design of yeast strains for novel flavor and fragrance production. Nevertheless, the practical use of genome-edited yeasts faces important challenges. Regulatory requirements, ecological risks in open environments, and consumer acceptance remain significant barriers. Future work should therefore prioritize biosafety assessment, traceability, and validation of strain performance under application-specific conditions.

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