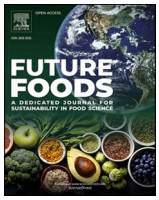
Future Foods 12 (2025) 100752

Contents lists available at ScienceDirect

Future Foods

journal homepage: www.elsevier.com/locate/fufo



Yeast-based precision fermentation for the biosynthesis of terpenoids

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ARTICLE INFO

Keywords:
Terpenoid
Yeast
Precision fermentation
Synthetic biology

ABSTRACT

Terpenoids represent the largest class of specialized metabolites with extensive applications in food and nutraceutical industries. While plant-derived extraction remains the predominant industrial source, this traditional approach faces significant challenges including low yields, environmental variability, and substantial resource requirements. Yeast-based precision fermentation has emerged as a compelling alternative production platform, effectively addressing these constraints through distinct advantages including rapid growth kinetics, environmental condition independence, and superior genetic tractability. This review scrutinizes recent developments in yeast-based precision fermentation for terpenoid production, with emphasis on food-related applications. We discuss key yeast platforms such as *Saccharomyces cerevisiae, Komagataella phaffii, Yarrowia lipolytica*, and *Candida tropicalis*, analyze their distinct advantages as production hosts, and explore synthetic biology approaches for strain development. Through representative case studies of mono-, sesqui-, di-, and triterpenoid production, we illustrate the practical implementation of the synthetic biology approaches and highlight exceptional achievements in production metrics. Finally, this review examines emerging methodologies for expanding the repertoire of producible terpenoids through functional metagenomic screening and synthetic pathway construction for noncanonical building blocks. This comprehensive analysis provides insights into the current state and future directions of yeast-based precision fermentation for sustainable terpenoid production.

1. Introduction

Terpenoids represent the largest and structurally diverse class of specialized metabolites, comprising over 80,000 characterized compounds (Rudolf et al., 2021). These natural products demonstrate exceptional food ingredient market value due to their broad biological activities, including distinctive organoleptic properties and antioxidative capacity, that are particularly valuable in food and nutraceutical applications (Câmara et al., 2024; Caputi and Aprea, 2011). While terpenoids are biosynthesized across various organisms, including fungi and bacteria, plant-derived sources remain the predominant commercial source for terpene production (Câmara et al., 2024; Chang and Keasling, 2006).

However, traditional plant-based extraction methodologies face numerous significant challenges that constrain industrial-scale production. A primary limitation is the inherently low biosynthetic yield in natural sources, where these specialized metabolites typically constitute only a marginal fraction of total dry biomass, necessitating the processing of substantial quantities of plant material to achieve commercially viable production scales (Chang and Keasling, 2006; Mewalal et al., 2017; Zhang and Hong, 2020). The biosynthesis of these compounds in natural sources is subject to significant environmental influences, resulting in significant variability in both yield and compositional profiles. Environmental parameters, including seasonal fluctuations, climatic conditions, and various environmental stressors, significantly impact metabolite accumulation patterns and chemical compositions. These variations are increasingly exacerbated by climate change phenomena, which influence plant growth patterns and secondary metabolite biosynthesis through multiple physiological mechanisms (Li et al., 2020; Qaderi et al., 2023). Also, traditional extraction methodologies present substantial resource-related challenges. Industrial-scale production necessitates extensive agricultural land allocation, considerable water resources for cultivation, and prolonged growth cycles prior to harvest. These requirements contribute to significant operational costs and environmental impact (Chang and Keasling, 2006; Mewalal et al., 2017). Furthermore, the technical complexity

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https://doi.org/10.1016/j.fufo.2025.100752
Received 30 January 2025; Received in revised form 30 August 2025; Accepted 2 September 2025
Available online 4 September 2025
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of extraction and purification processes, requiring sophisticated protocols and specialized equipment, results in extended production timelines and substantial capital investment requirements, ultimately affecting scalability (Bureau et al., 2023; Mewalal et al., 2017).

These multifaceted constraints have accelerated the exploration of alternative production strategies, particularly microbial-based precision fermentation systems, for the production of terpenoids. Precision fermentation offers a more sustainable and scalable production platform through the introduction of terpenoid synthases and optimization of metabolic pathways in microbial hosts (Bureau et al., 2023; Chang and Keasling, 2006). Microbial systems, including fungi and bacteria, present distinct advantages including rapid growth kinetics, environmental condition independence, and superior genetic tractability. These characteristics contrast advantageously with plant-based systems, which present significant limitations for synthetic biology applications due to their slow growth cycles, inefficient transformation protocols, and complex metabolic networks (Cross et al., 2017; Jaramillo-Madrid et al., 2022; Liu et al., 2022). The present review focuses on representative yeast host systems and precision fermentation strategies employed in microbial terpenoid production for food applications. Special emphasis is placed on state-of-the-art developments in yeast-based precision fermentation for terpenoid production, illustrated through case studies of food-related terpenoid production using engineered yeasts.

2. Yeast as a host of precision fermentation in terpenoid biosynthesis

Yeasts are unicellular fungal microorganisms exhibiting exceptional tolerance to adverse conditions prevalent in industrial fermentation processes, including acidic pH, elevated osmotic pressure, and bacteriophage contamination (Segal-Kischinevzky et al., 2022). These inherent advantages make yeasts ideal industrial hosts for the production of diverse biomolecules. Yeasts have additional benefits as eukaryotic organisms over bacterial hosts especially for the biosynthesis of terpenoids. First, as eukaryotic organisms, yeasts possess cellular environments and machinery that more closely resemble those of plants, making them preferred hosts for the heterologous expression of eukaryote-derived enzymes compared to bacteria (Van Gelder et al., 2024). This characteristic presents a substantial advantage, given that terrestrial and marine plants, liverworts, and fungi represent the primary sources of characterized terpenoids, whereas prokaryotes contribute only minimally to the known diversity (Yamada et al., 2015). Second, yeasts utilize the mevalonate (MVA) pathway to synthesize terpene precursors, namely prenyl pyrophosphates (Fig. 1A). This metabolic pathway has proven more effective than the bacterial 1-deoxy-d-xylulose 5-phosphate (DXP) pathway (also called the 2-C-methyl-d-erythritol phosphate pathway) (Paddon and Keasling, 2014; Wang et al., 2018). Third, its capacity to produce and store metabolites within cellular compartments can be particularly beneficial for

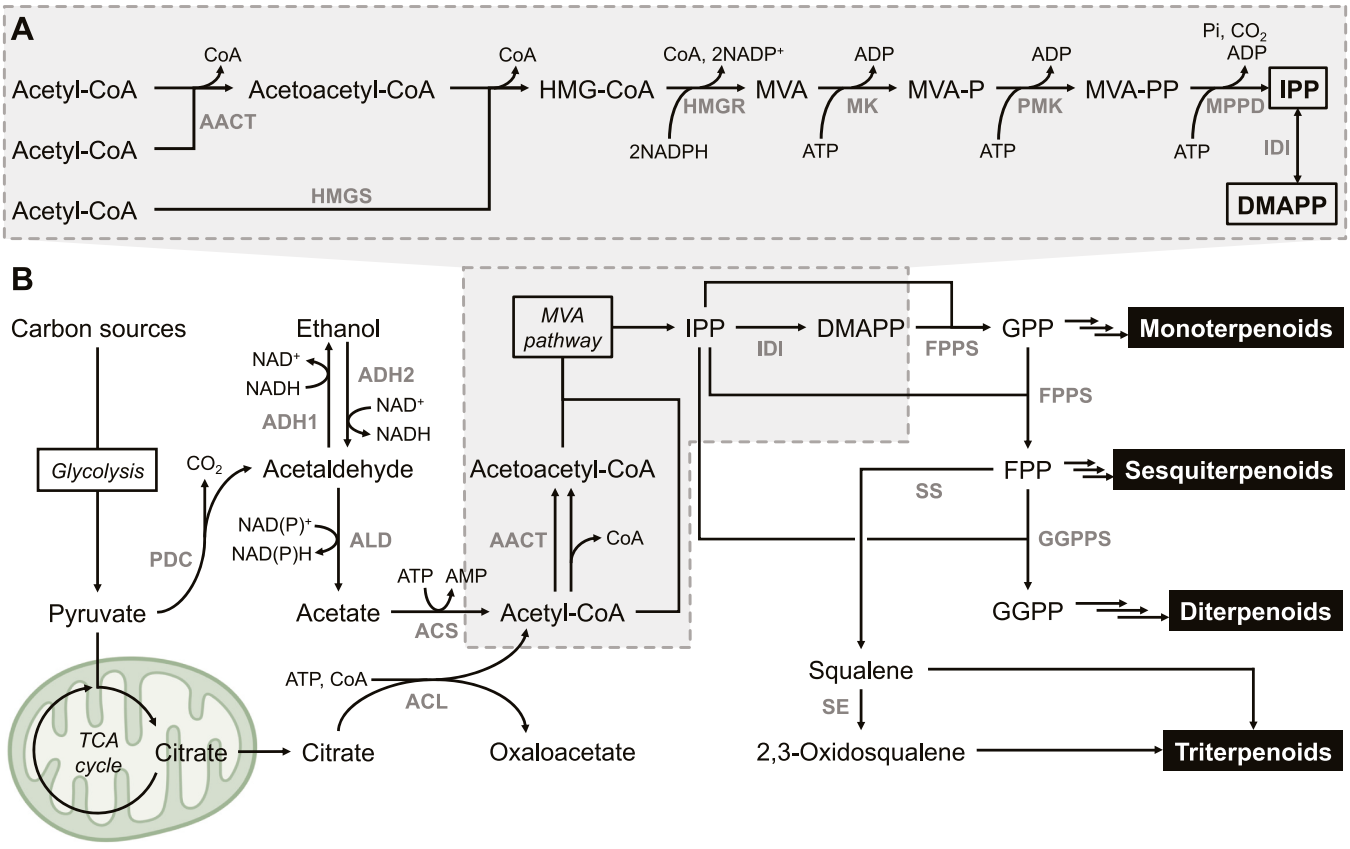

Fig. 1. Terpenoid biosynthesis in native and engineered yeasts. (A) The mevalonate pathway for biosynthesizing the first prenyl pyrophosphate intermediates, isopentenyl pyrophosphate and dimethylallyl pyrophosphate. (B) Scheme of biosynthetic routes for precursors of terpenoids. Metabolites: CoA, coenzyme A; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; MVA-P, mevalonate phosphate; MVA-PP, mevalonate pyrophosphate; DMAPP, dimethylallyl pyrophosphate; IPP, isopentenyl pyrophosphate; GPP, geranyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NADP+, nicotinamide adenine dinucleotide phosphate. Enzymes: AACT, acetyl-CoA C-acetyltransferase; HMGS, HMG-CoA synthase; HMGR, HMG-CoA reductase; MK, mevalonate kinase; PMK, phosphomevalonate kinase; MPPD, mevalonate pyrophosphate decarboxylase; IDI, isopentenyl diphosphate: dimethylallyl diphosphate isomerase; PDC, pyruvate decarboxylase; ADH1, alcohol dehydrogenase; ADH2, ethanol-oxidizing alcohol dehydrogenase; ALD, aldehyde dehydrogenase; ACS, acetyl-CoA synthetase; ACL, ATP-citrate lyase; AACT, acetyl-CoA C-acetyltransferase; IDI, isopentenyl diphosphate:dimethylallyl diphosphate isomerase; FPPS, farnesyl pyrophosphate synthase; GGPPS, geranylgeranyl pyrophosphate synthase; SS, squalene synthase; SE, squalene epoxidase.

compounds like terpenes and lipids. This compartmentalization can protect sensitive molecules and allow for higher product accumulation without toxic effects on cell growth (Liu et al., 2020; Wang et al., 2024; Wei et al., 2021). The compartmentalization ability of yeast enables strategic spatial organization of terpenoid biosynthetic pathways across organelles, optimizing metabolic efficiency while maintaining cellular homeostasis (see Section 3.2).

Additionally, yeasts demonstrate superior capacity for both the biosynthesis and intracellular accumulation of terpene precursors than *Escherichia coli*, the most representative and genetically amenable prokaryotic host. Although *E. coli* can achieve enhanced terpene derivative production through overexpression of either the native DXP pathway or the heterologous MVA pathway as well (Ajikumar et al., 2010; Martin et al., 2003; Peralta-Yahya et al., 2011), excessive accumulation of prenyl pyrophosphates significantly inhibits the growth of *E. coli* eventually (Kwak et al., 2022; Martin et al., 2003). Lastly, yeasts present fewer safety and public perception concerns compared to *E. coli* when used as host microbes in precision fermentation for food production (Vieira Gomes et al., 2018). Most notably, yeasts lack lipopolysaccharide endotoxins, which are present in *E. coli* and can make it unsuitable for food applications (Taguchi et al., 2015). Even non-pathogenic laboratory strains of *E. coli* require more stringent containment and purification processes to ensure complete removal from final food products (Lopes et al., 2010; Mamat et al., 2015). Still, commercial terpenoid production for food-grade applications through yeast-based precision fermentation may be subject to regulatory constraints depending on the Generally Recognized as Safe (GRAS) status of the microbial host organism. The following sections present representative yeast strains that have emerged as microbial platforms for the biosynthesis of terpenes and their derivatives via precision fermentation methodologies. Key characteristics of the yeast platforms were compared in Table 1.

2.1. *Saccharomyces cerevisiae*

S. cerevisiae stands as a representative yeast platform in industrial fermentation and presents numerous advantages as a prominent host organism for precision fermentation-based food material production. As a GRAS organism with a long history in food fermentation, *S. cerevisiae* has widespread acceptance in the food industry and a favorable public perception. Its extensive commercial use in traditional food products such as bread, wine, and beer has established a comprehensive safety profile through human consumption without adverse effects (Long et al., 2024; Vieira and Delerue-Matos, 2020). In addition, *S. cerevisiae* possesses robust cellular machinery for protein expression and post-translational modifications as well as metabolic potential to produce varied value-added biochemicals, making it suitable for producing complex food ingredients (Guo et al., 2024; Vieira and Delerue-Matos, 2020). As a model eukaryotic microorganism, *S. cerevisiae* also has an extensively characterized genomic information and well-established genetic tools, facilitating precise metabolic engineering for the optimized production. Still, *S. cerevisiae* has a noticeable metabolic limitation as a host for precision fermentation of terpenes and their derivatives as a Crabtree-positive yeast. When cultivated on fermentable sugars, including glucose—the primary carbon source in fermentation industries—Crabtree-positive yeasts exhibit a metabolic stance predominantly oriented toward ethanol fermentation, irrespective of oxygen availability (Gambacorta et al., 2020; Malina et al., 2021). This metabolic predisposition, namely glucose repression, causes a considerable disadvantage for the biosynthesis of non-ethanol metabolites, which require substantial energy investment for their production, such as terpene molecules and derivatives (Kwak et al., 2019).

Table 1
Representative yeast platforms for microbial production of terpenoids.

Species	GRAS status	Advantage	Disadvantage
S. cerevisiae	Yes	Long history in food fermentation, extensive genetic tools, favorable perception for food applications	Crabtree-positive (excessive accumulation of ethanol, energy inefficiency)
K. phaffii	Partial GRAS (strain specific)	Crabtree-negative, cost-effective substrates (fatty acid and methanol),	Fewer established protocols and optimization strategies
Y. lypolytica	Partial GRAS (strain specific)	Oleaginous, high cytosolic acetyl-CoA availability, robust cofactor supply (NADPH and ATP)	Challenges in secretion of hydrophobic compounds, complex metabolic engineering
C. tropicalis	No	Oleaginous, physiological robustness in industrial fermentation	Causative agent of candidiasis, limited research history

2.2. *Komagataella phaffii (Pichia pastoris)*

Although *K. phaffii* has established itself as a robust industrial platform for heterologous protein production, its potential as a host organism for terpene biosynthesis remained relatively unexplored until recent investigations (Liu et al., 2021; Ye et al., 2024; Zuo et al., 2022). It exhibits distinct metabolic characteristics that differentiate it from *S. cerevisiae*, most notably its Crabtree-negative phenotype. This metabolic trait indicates that *K. phaffii* primarily relies on respiratory metabolism even in the presence of relatively higher glucose concentrations, contrasting with the fermentative metabolism observed in *S. cerevisiae* (Baumann et al., 2010). *K. phaffii* also demonstrates distinct advantages in bioprocess economics through its capacity to utilize cost-effective carbon sources, including fatty acids and methanol, while achieving high cell density fermentation. These characteristics facilitate economically favorable scale-up processes compared to other conventional yeasts including *S. cerevisiae* (Liu et al., 2021). Although multiple product-specific GRAS notifications have been granted for *K. phaffii*, in contrast to *S. cerevisiae, K. phaffii* lacks species-wide GRAS determination (Kastberg et al., 2022).

2.3. *Yarrowia lypolytica*

Y. lipolytica is a Crabtree-negative, genetically tractable yeast demonstrating robust capacity for heterologous enzyme expression and secretion (Vandermies and Fickers, 2019). *Y. lipolytica* possesses versatile metabolic capabilities, efficiently utilizing diverse carbon sources through its active tricarboxylic acid cycle and pentose phosphate pathway. A distinguishing characteristic of *Y. lipolytica* is its substantial lipid accumulation capacity, which facilitates the intracellular storage of hydrophobic compounds that typically present secretion challenges (Zhang et al., 2022). This trait is particularly advantageous to produce complex and high-molecular-weight terpenes and terpenoids (>C30), typically requiring intracellular accumulation (Wei et al., 2021). This oleaginous yeast maintains exceptionally high cytosolic acetyl-CoA concentrations, the critical precursor for the MVA pathway. This metabolic feature results from the robust tricarboxylic acid cycle and pentose phosphate pathway activities, which support elevated fluxes of acetyl-CoA, NADPH, and ATP when metabolizing various carbon substrates (Muhammad et al., 2020; Zhang et al., 2022). Multiple *Y. lipolytica* strains have received GRAS status, establishing their suitability for applications in food additive and nutraceutical production (Groenewald et al., 2014). However, this designation is strain-specific rather than species-level, which creates regulatory limitations for food industry applications.

2.4. *Candida tropicalis*

C. tropicalis also belongs to the group of oleaginous yeasts. Its natural

propensity as an oleaginous yeast is well-suited for accumulating lipids and synthesizing hydrophobic secondary metabolites, particularly terpenes and terpenoids, through both native and engineered metabolic pathways. Recent advances in molecular biology have established robust genetic manipulation protocols for *C. tropicalis*, including the implementation of CRISPR-Cas9-mediated genome editing systems (Gao et al., 2021). An important characteristic of *C. tropicalis* is its remarkable physiological robustness in industrial fermentation environments. It demonstrates notable tolerance to various stress factors commonly encountered in large-scale bioprocesses (Kim et al., 2019; Thangavelu et al., 2020). This inherent stress resistance, coupled with its metabolic versatility utilizing various substrates and product formation capabilities, establishes *C. tropicalis* as another promising platform yeast for precision fermentation applications for terpene biosynthesis. Nevertheless, *C. tropicalis* presents significant regulatory and safety limitations as a production platform for food-grade terpenoids. Unlike other yeast species discussed, *C. tropicalis* lacks a GRAS designation and is considered an opportunistic pathogen associated with invasive candidiasis, particularly in immunocompromised populations (Xu, 2021).

2.5. Trade-offs of platform selection for food-grade terpenoid production

The species-level GRAS designation of *S. cerevisiae* confers a critical competitive advantage over other microbial hosts for terpenoid production in food applications. This regulatory status enables flexible host engineering including genetic modifications without necessitating individual regulatory approval for each genetic variant, thereby providing substantial operational and economic advantages for food industrial applications (Elhalis, 2024; Gao et al., 2020; Parapouli et al., 2020). Beyond regulatory considerations, *S. cerevisiae* offers the additional benefits of extensive genomic characterization, well-established genetic tools, and widespread public acceptance stemming from its long association with food production. Conversely, yeasts requiring strain- and product-specific GRAS determinations, namely *K. phaffii* and *Y. lipolytica*, necessitate separate regulatory approval processes, substantially increasing compliance costs and extending approval timelines (EFSA Panel on Food Additives and Flavourings (FAF) et al., 2023). These regulatory constraints may impede the commercial viability of *K. phaffii*- and *Y. lipolytica*-based terpenoid production for food applications. The regulatory burden becomes particularly pronounced when considering the iterative nature of strain development, where each genetic modification cycle could potentially require renewed regulatory assessment.

While *S. cerevisiae* remains the gold standard for food-grade applications due to its established GRAS status and regulatory familiarity, emerging evidence suggests that optimal platform selection should be compound-specific. The Crabtree-negative phenotype of *K. phaffii* and *Y. lipolytica* presents distinct advantages for terpenoid production, potentially offering superior yields for energy-intensive biosynthetic pathways compared to the ethanol-fermenting *S. cerevisiae*. In addition, the respiratory metabolism and methanol utilization capability of *K. phaffii* offer unique advantages for cost-effective large-scale production, particularly when utilizing alternative carbon sources. In the case of *Y. lipolytica*, its exceptional acetyl-CoA concentrations and intracellular lipid storage capacity make it ideally suited for triterpenes and complex terpenoids, while *S. cerevisiae*'s glucose repression may limit its efficiency for terpenes requiring substantial ATP and NADPH investment. On the other hand, excluding *C. tropicalis* from food-grade applications should be an important regulatory constraint to consider for selecting a platform yeast, despite its promising maintenance properties and stress tolerance.

3. Metabolic engineering and bioprocess optimization of yeast for terpenoid production

The optimization of terpene biosynthesis in yeast-based precision fermentation encompasses multiple strategic engineering approaches, ranging from pathway-level modifications to environmental modulation. This section demonstrates five fundamental strategies for optimizing the terpene production efficiency of precision fermentation based on the exemplar yeast species. These approaches address the multifaceted challenges collectively and complementarily in optimizing terpene production capacity while maintaining cellular homeostasis in engineered yeast strains.

3.1. Optimization of MVA pathway and terpenoid backbone biosynthesis

The MVA pathway serves as the biosynthetic source of essential prenyl pyrophosphates—specifically isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP)—which function as fundamental precursors in downstream terpenoid biosynthesis (Fig. 1). Thus, MVA pathway engineering has been considered as the predominant focus in yeast-based precision fermentation strategies for the terpene production. Yeasts initiate the MVA pathway with the enzymatic condensation of two cytosolic acetyl-CoA molecules by acetoacetyl-CoA thiolase (acetyl-CoA C-acetyltransferase, AACT), yielding acetoacetyl-CoA, which undergoes subsequent enzymatic modification to generate 3-hydroxy-3-methylglutaryl CoA (HMG-CoA). HMG-CoA reductase (HMGR) mediates the conversion of HMG-CoA to mevalonate. Mevalonate undergoes a series of successive phosphorylation and decarboxylation events, ultimately generating IPP and DMAPP (Fig. 1A) (Dinday and Ghosh, 2023; Guo et al., 2024). The HMGR, which catalyzes the rate-limiting reaction of this biosynthetic sequence, represents the primary target for genetic manipulation to enhance metabolic flux through the MVA pathway (Guo et al., 2024). Strategic implementation of truncated HMGR without the membrane-binding domain has been preferentially employed as an overexpression target instead of the native intact enzyme, thereby circumventing ergosterol-mediated feedback regulation and enhancing its catalytic activity (Lu et al., 2022; Xia et al., 2022). AACT catalyzing the initial condensation reaction of the MVA pathway is another important target for overexpression to effectively channel cytosolic acetyl-CoA flux toward MVA pathway intermediates (Kwak et al., 2019). The optimization of this enzymatic step assumes particular significance when implemented in conjunction with metabolic engineering strategies designed to augment cytosolic acetyl-CoA availability (Arnesen et al., 2020; Kwak et al., 2019).

Farnesyl pyrophosphate synthase (FPPS) after the MVA pathway is another critical control point, catalyzing the sequential condensation of IPP with DMAPP to form GPP, followed by the addition of another IPP molecule to produce FPP (Fig. 1B). As GPP and FPP serve as precursors for monoterpene and sesquiterpene biosynthesis, respectively, precise modulation of FPPS activity is crucial, particularly for enhancing monoterpene production in yeasts (Ren et al., 2020; Zhu et al., 2021). To this end, a dominant negative FPPS variant ($ERG20^{F96W/N127W}$) that preferentially synthesizes GPP while minimizing FPP production was created (Fig. 2A). The F96W mutation significantly reduces GPP binding affinity (30-fold) while maintaining DMAPP affinity, effectively maximizing GPP availability. This effect is complemented by the N127W mutation, which exploits the dimeric nature of FPPS where N127 from one subunit influences the active site of its partner, thereby enhancing the dominant negative phenotype (Ignea et al., 2014). Geranylgeranyl pyrophosphate (GGPP) serves as the precursor for diterpenoid biosynthesis and is synthesized through the condensation of IPP and FPP by GGPP synthase (GGPPS, Fig. 1B). Inefficient substrate channeling between FPPS and GGPPS can cause FPP accumulation, leading to feedback inhibition of upstream enzymes or diversion of precursors toward competing metabolic pathways. Therefore, the efficient coupling of FPPS and GGPPS enzymatic reactions represents a critical requirement for enhancing diterpenoid production in yeast systems. This coupling has been successfully implemented through direct enzyme fusion of FPPS and GGPPS, and utilization of a FPPS variant ($ERG20^{F96C}$) capable of producing GGPP from FPP (Fig. 2C). Both approaches have

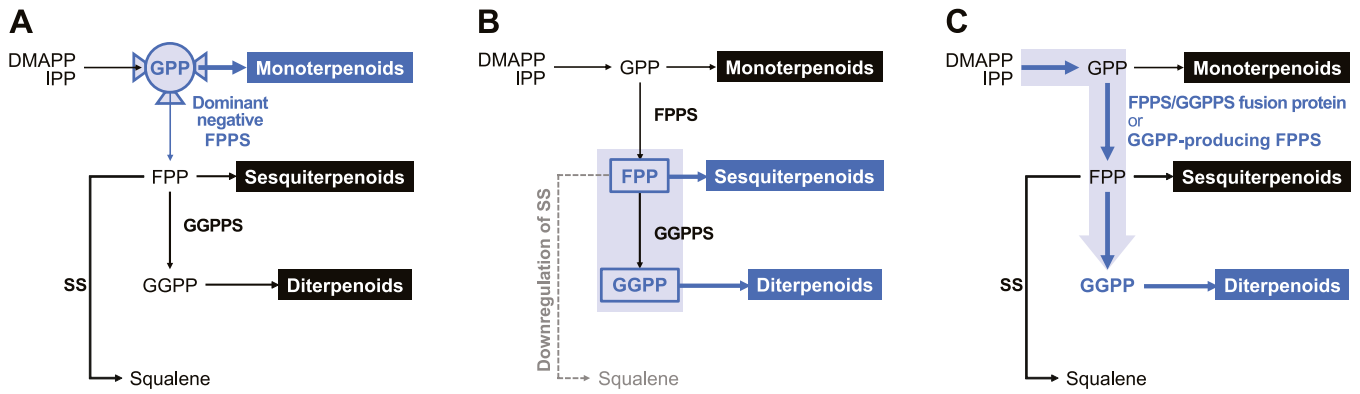

Fig. 2. Metabolic engineering for enhancing the availability of target prenyl pyrophosphates in yeasts. (A) Dominant negative farnesyl pyrophosphate synthase (FPPS) is a catalytically modified variant of FPPS ($ERG20^{F96W/N127W}$) that functions as a regulator, selectively promoting geranyl pyrophosphate (GPP) biosynthesis while minimizing the original FPP biosynthetic activity, consequently allowing efficient production of monoterpenoids. (B) Downregulation of squalene synthase (SS) increases the availability of both FPP and geranylgeranyl pyrophosphate (GGPP), backbones of sesquiterpenoids and diterpenoids, accordingly enhances the production of corresponding terpenoids. (C) Coupling of FPPS and GGPP synthase (GGPPS) activities—either through enzyme fusion or by using a GGPP-producing FPPS variant ($ERG20^{F96C}$)—leads to improved diterpenoid production via efficient substrate channeling toward GGPP.

demonstrated significant improvements in diterpenoid yields in yeast platforms (Ignea et al., 2015; Ohto et al., 2010; Zhou et al., 2012).

3.2. Compartmentalization

The orchestrated spatial organization of biosynthetic pathways for terpenes and derivatives is a sophisticated approach to optimize metabolic efficiency while simultaneously maintaining cellular homeostasis. Eukaryotic cells can produce and store terpenes and derivatives through their diverse organelles and membrane structures, including the endoplasmic reticulum (ER), Golgi complex, lipid droplets, peroxisomes, mitochondria, and plasma membrane (Jaramillo-Madrid et al., 2022). Recent advances in synthetic biology have enabled innovative engineering of subcellular organelles for optimized terpene biosynthesis (Yanagibashi et al., 2024; Zhang et al., 2024). If the accumulation of target terpene compounds is toxic to cells, compartmentalization of the synthetic processes across different organelles in yeasts not only enhances the catalytic efficiency of corresponding enzymes but also protects yeast cells from the toxic effects (Jin et al., 2022). The strategic compartmentalization of biosynthetic pathways also facilitates spatial segregation of metabolic processes, mitigating substrate and cofactor competition between endogenous and heterologous pathways while enabling localized substrate concentration for enhanced reaction kinetics (Jaramillo-Madrid et al., 2022). Furthermore, the multi-compartment engineering approach can maximize the utilization of spatial capacities as well as cellular resources for terpene biosynthesis (Zhang et al., 2020).

Specifically, peroxisomes and the ER serve as primary engineering targets for enhancing the efficiency of terpenoid biosynthesis in yeasts. Peroxisomes are organelles bounded by a single bilayer membrane and serve as effective subcellular factories, particularly in non-oleophilic yeasts. Peroxisomes have demonstrated significant utility as subcellular compartments for terpene biosynthesis in yeast systems. Yeast peroxisomes primarily function in peroxide decomposition, fatty acid β-oxidation, the glyoxylic acid cycle, and methanol metabolism, thereby providing essential precursors and cofactors for terpene biosynthesis (Ravindran et al., 2023; Zhang et al., 2024). On the other hand, ER compartmentalization is critical for the functional expression of membrane-localized terpenoid biosynthetic pathway components, such as cytochrome P450s (Fig. 3A) (Arendt et al., 2017; Kim et al., 2019). Engineering strategies for ER compartmentalization have predominantly focused on expanding ER volume in yeasts through either deletion of the phosphatidic acid phosphatase-encoding gene PAH1 or upregulation of the ER size regulatory factor-encoding INO2,

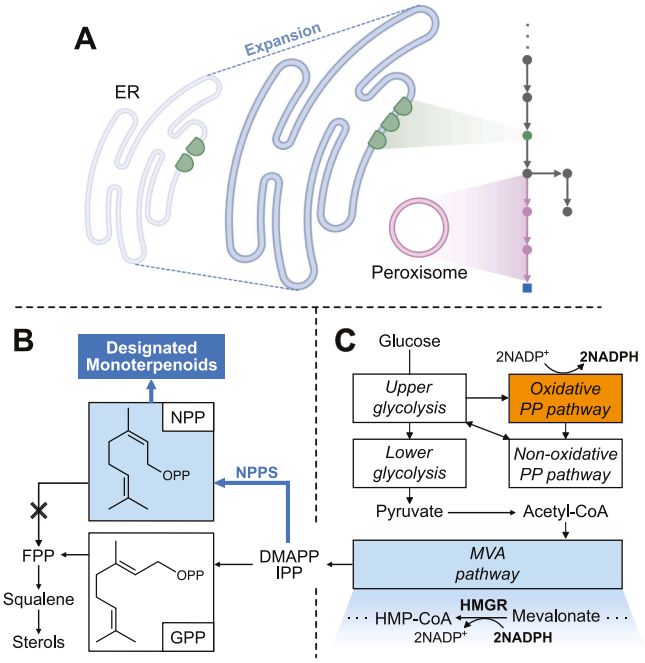

Fig. 3. Scheme of yeast engineering strategies for efficient terpenoid production. (A) The endoplasmic reticulum (ER) and peroxisome serve as key organelles for compartmentalizing terpenoid biosynthetic pathways. The ER is particularly effective for expressing membrane-localized enzymes (green), with enhanced performance achievable through volume expansion. (B) Introduction of neryl pyrophosphate synthase (NPPS) creates a metabolic branch point for synthetic monoterpenoid pathways. The synthetic pathways are orthogonal to native metabolism since NPP, a *cis*-isomer of GPP, cannot be utilized by endogenous GPP-dependent pathways. (C) Redirecting glycolytic flux through the pentose phosphate (PP) pathway enhances terpenoid biosynthesis in Crabtree-positive yeasts by efficiently regenerating NADPH, the essential coenzyme for HMG-CoA reductase (HMGR), the key enzyme of the mevalonate (MVA) pathway.

approaches that have yielded substantial improvements in terpene production (Arendt et al., 2017; Kim et al., 2019). Overexpression of *ICE2*, which encodes an ER transmembrane protein, represents a promising strategy demonstrated to enhance both the volume and surface area of the ER through promotion of ER membrane biosynthesis

(Papagiannidis et al., 2021). Notably, when combined with optimization of ER-associated terpenoid biosynthetic pathways, additional *ICE2* overexpression has been shown to significantly enhance the biosynthesis of target terpenoid molecules in yeast hosts (Emmerstorfer et al., 2015; Zhang et al., 2018).

3.3. Orthogonal synthetic pathway

The establishment of synthetic pathways, which is utterly independent from innate cellular activities, represents an advanced alternative to conventional metabolic engineering strategies depending on native biochemical elements. Specifically, orthogonal synthetic pathway offers advantages including minimized metabolic competition with native pathways, enhanced flux controllability, and reduced interference with essential cellular processes (Chou et al., 2021; Pandit et al., 2017). A pioneering study by Ignea et al. demonstrated the first successful implementation of an orthogonal terpene biosynthetic pathway from neryl pyrophosphate (NPP), the *cis*-isomer of GPP (Fig. 3B), in *S. cerevisiae* (Ignea et al., 2019). NPP biosynthesis is catalyzed by NPP synthase from *Solanum lycopersicum*, which mediates the condensation of DMAPP and IPP in a *cis*-configuration-specific manner (Schilmiller et al., 2009). NPP circumvents the native sterol biosynthetic pathway mediated by FPPS in *S. cerevisiae*, thereby enabling enhanced production of target terpene molecules through mitigation of substrate competition with endogenous metabolism and facilitation of precise dynamic regulation of the target biosynthetic pathway (Fig. 3B) (Ignea et al., 2019).

3.4. Cofactor engineering

The availability of reduced nicotinamide adenine dinucleotide phosphate (NADPH) in yeast is decisive for the terpene overproduction in yeasts. NADPH serves as an essential coenzyme in the MVA pathway, where the rate-limiting enzyme HMGR consumes two NADPH molecules to catalyze the conversion of one HMG-CoA molecule to mevalonate (Fig. 1) (Chen et al., 2023; Kwak et al., 2020). Moreover, NADPH plays multifaceted roles in yeast cellular metabolism; it is required for biomass accumulation, heterologous protein biosynthesis, and cellular defense mechanisms against both ER stress and oxidative stress, which are all crucial determinants for the successful precision fermentation (Chen et al., 2023; Tomàs-Gamisans et al., 2020). The oxidative pentose phosphate pathway (oxPPP) is the major inherent metabolic avenue regenerating NADPH from NADP+ in yeasts on glucose and other fermentable sugars. Consequently, metabolic engineering strategies that enhance oxPPP flux have proven effective in increasing cellular NADPH availability, thereby promoting terpenoid biosynthesis across various engineered yeast strains (Adusumilli et al., 2024; Chen et al., 2023; Kwak et al., 2020; Liu et al., 2024a). As oxPPP and glycolysis compete for glucose 6-phosphate as a shared metabolic intermediate, redirecting metabolic flux from glycolysis toward oxPPP creates a metabolic trade-off; it enhances NADPH availability and simultaneously reduces biomass generation (Kwak et al., 2020). This metabolic dichotomy necessitates careful optimization of flux distribution between glycolysis and oxPPP to achieve optimal terpene biosynthesis yields in yeast-based production systems (Fig. 3C). In addition, a complementary approach involves the introduction of heterologous NADH-dependent HMGR from *Silicibacter pomeroyi*, which reduces the MVA pathway's reliance on NADPH during terpene overproduction. This strategy effectively aligns the cofactor requirements of the MVA pathway with the cellular NAD(P)H environment, thus increasing the production of terpene compounds in yeasts (Meadows et al., 2016; Wang et al., 2023, 2021).

3.5. Environmental perturbation

Glucose repression is a significant challenge for terpenoid production in Crabtree-positive yeasts, as ethanol overproduction leads to the diminished yield of target terpenoids. It also results in suboptimal ATP generation, as fermentative metabolism yields significantly less energy than respiratory metabolism, conflicting with the high energy requirements of terpenoid biosynthesis. (Dai et al., 2018; Yiming Zhang et al., 2022). Furthermore, ethanol accumulation during cultivation reduces yeast viability and constrains yeast biomass titer, which particularly impacts the production of intracellular terpenoids (Kwak et al., 2017). Fed-batch fermentation is an effective approach to mitigate glucose repression in Crabtree-positive yeasts during glucose utilization. The controlled, gradual glucose feeding strategy prevents glucose repression, thereby minimizing ethanol accumulation while maintaining respiratory metabolism, ultimately enhancing biomass formation and terpenoid production yields (Carsanba et al., 2021).

Utilization of non-fermentable carbon sources represents an alternative strategy for circumventing glucose repression. Xylose is a representative non-fermentable sugar, which is the second most abundant sugar component of lignocellulosic biomass (Bolzico et al., 2024; Lee et al., 2021). While most yeast strains exhibit marginal or no native xylose assimilation capacity, extensive research has focused on metabolic engineering strategies to establish and enhance xylose utilization in yeasts. The initial motivation of the yeast engineering approaches was to bypass ethical concerns regarding the use of edible feedstocks, such as glucose, in biorefinery applications (Kwak et al., 2019; Lee et al., 2021). However, subsequent studies have revealed that xylose metabolism confers significant advantages to produce non-ethanol metabolites, including terpenes, particularly in Crabtree-positive yeasts such as *S. cerevisiae*. The reduction of expression levels of hexokinase 2, an enzyme that performs dual functions in glucose phosphorylation and transcriptional regulation mediating glucose repression, represents a critical component of the beneficial xylose effect in *S. cerevisiae* (Kwak et al., 2019). This transcriptional modulation leads to the de-repression of genes controlling mitochondrial respiratory metabolism, specifically those encoding components of the tricarboxylic acid cycle and oxidative phosphorylation pathways (Fig. 4) (Kwak et al., 2017; Lee et al., 2021). Furthermore, xylose utilization increases transcriptional levels of pathways involved in cytosolic acetyl-CoA biosynthesis, a crucial substrate for the MVA pathway (Fig. 1A). In particular, xylose attenuates glucose repression on the cytosolic acetyl-CoA synthetic pathway in S. cerevisiae, including aldehyde dehydrogenases and acetyl-CoA synthetase 1, as well as ethanol-oxidizing alcohol dehydrogenase (Fig. 4) (Kwak et al., 2019, 2017). The concurrent enhancement of both energy metabolism and cytosolic acetyl-CoA availability synergistically augments terpene biosynthesis in Crabtree-positive yeasts, such as *S. cerevisiae* and *Phaffia rhodozyma*, cultivated on xylose compared to glucose (Kwak et al., 2017; Montanti et al., 2011).

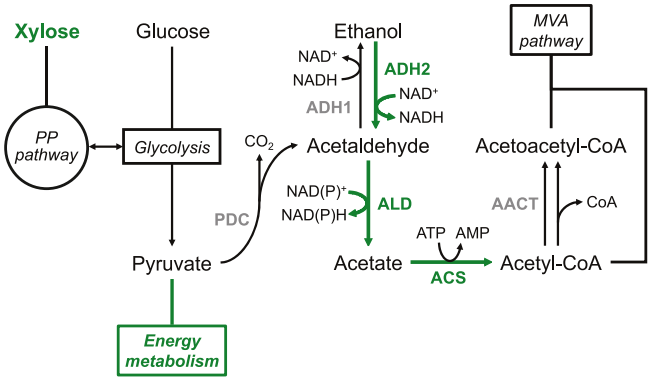

Fig. 4. Metabolic changes in Crabtree-positive yeast during xylose utilization. Metabolic pathways highlighted in green show higher transcription levels during growth on xylose compared to glucose. PDC, pyruvate decarboxylase; ADH1, alcohol dehydrogenase; ADH2, ethanol-oxidizing alcohol dehydrogenase; ALD, aldehyde dehydrogenase; ACS, acetyl-CoA synthetase; AACT, acetyl-CoA C-acetyltransferase.

4. Production of terpenoids via yeast-based precision fermentation

The subsequent sections describe recent advances in terpenoid production via yeast-based precision fermentation, with emphasis on food ingredients and nutraceuticals. The sections encompass developments in the biosynthesis of monoterpenoids (C10), sesquiterpenoids (C15), diterpenoids (C20), and triterpenoids (C30). Tetraterpenoids (C40), predominantly represented by carotenoids in nature, are excluded from this discussion as their production via yeast precision fermentation has been extensively reviewed elsewhere (Guo et al., 2024; Kanamoto et al., 2021; Mussagy, 2024; Naz et al., 2023; Sandmann, 2022; Vargas-Sinisterra and Ramírez-Castrillón, 2021; Zhang et al., 2023). Through case studies organized by carbon number classification, we illustrate the practical implementation of various engineering strategies we described in Section 3, with particular emphasis on strain designs demonstrating exceptional production metrics or pioneering engineering approaches in microbial terpene biosynthesis. The key achievements of the studies discussed below are summarized in Table 2.

4.1. Monoterpenoids

Monoterpenoids are biosynthetically derived from geranyl pyrophosphate (GPP), the universal precursor of monoterpene biosynthesis in yeasts. GPP is synthesized through the condensation of DMAPP and IPP after the MVA pathway. Monoterpenes and their derivatives exhibit various skeletal arrangements including acyclic, monocyclic, and bicyclic frameworks (Zielińska-Błajet and Feder-Kubis, 2020). These compounds are widely used for flavoring, fragrances, colorants, and functional ingredients in the food and beverage industries (Zhu et al., 2021).

Limonene is a prototypical monoterpene example that exhibits industrial applications in the food and beverage sectors where it serves as a critical organoleptic compound contributing characteristic citrus notes to various formulations (Ren et al., 2020). Limonene also exhibits several potential health benefits, including anti-inflammatory, antioxidant, and anticancer properties (Eddin et al., 2021). *S. cerevisiae* has emerged as the preeminent microbial platform for limonene production through precision fermentation, achieving superior titers compared to other yeast species and bacterial hosts (Li et al., 2021). Recent research in *S. cerevisiae* engineering for limonene production has achieved significant progress by moving beyond traditional metabolic engineering approaches, focusing instead on creating chemically orthogonal pathways to maximize limonene biosynthesis using NPP. The orthogonal pathway from NPP exhibited superior control characteristics and enhanced monoterpene production capabilities, achieving a limonene titer of 167.38 ± 20.88 mg/L in engineered *S. cerevisiae* expressing a limonene synthase (Ignea et al., 2019). This orthogonal pathway demonstrated compatibility with established metabolic engineering strategies for enhancing cytosolic acetyl-CoA availability. Notable approaches include the expression of a feedback-resistant variant of acetyl-CoA synthase from *Salmonella enterica* ($SeACS^{L641P}$) and the deletion of peroxisomal citrate synthase 2, which catalyzes the condensation of oxaloacetate and acetyl-CoA (Nielsen, 2014; Wegner et al., 2021; Zhang et al., 2021). The synergistic implementation of these interventions in conjunction with the NPP-based orthogonal pathway resulted in significant improvements in limonene production with 2.23 g/L of maximum titer in fed-batch fermentation (Zhang et al., 2021). The oleaginous yeast *Y. lipolytica* has emerged as a promising alternative platform for limonene production through yeast precision fermentation, with multiple studies demonstrating the effectiveness of peroxisomal compartmentalization for terpene biosynthesis (Arnesen et al., 2020; Ma et al., 2024). The targeting of the complete MVA pathway toward GPP to the peroxisome significantly enhanced limonene production, achieving a titer of 47.8 mg/L in flask cultivation. Through optimization of fed-batch fermentation conditions, the limonene titer was further improved to 69.3 mg/L (Park et al., 2024). On the other hand, limonene toxicity significantly impairs cellular fitness in yeasts, including *Y. lipolytica*, presenting a major challenge for improving production titers (Ren et al., 2020). Through transcriptomic analyses, Li et al. identified 82 genes upregulated in *Y. lipolytica* during limonene exposure, with 8 of these genes conferring enhanced limonene tolerance when overexpressed. Subsequent co-expression studies with limonene synthase demonstrated that 5 of the 8 tolerance-conferring genes improved

Table 2
Key achievements in terpenoid production via yeast-based precision fermentation presented in this review.

Category	Terpenoid	Food applications	Titer	Host	Key engineering approaches	Type	Reference
Monoterpenoid (C10)	Limonene	Flavoring agent (fruity)	2.23 g/L	*S. cerevisiae*	Increase in cytosolic acetyl-CoA supply, introduction of orthogonal limonene synthetic pathway	Fed-batch	Zhang et al., 2021
	β-Myrcene	Flavoring agent (beer)	142.64 mg/L	*S. cerevisiae*	Peroxisomal compartmentalization, enzyme fusion with dominant negative FPPS	Fed-batch	Shu et al., 2024
	Linalool	Flavoring agent (floral)	142.88 mg/L	*S. cerevisiae*	Introduction of dominant negative FPPS and isopentenol utilization pathway	Batch	Yaoyao Zhang et al., 2022
Sesquiterpenoid (C15)	β-Farnesene	Flavoring agent (fruity and floral), precursor of vitamin E and K1	3.09 g/L	*K. phaffii*	Increase in NADPH regeneration and ATP pool	Batch	Chen et al., 2023
	α-Humulene	Flavoring agent (woody and spicy), anti-inflammatory agent	17.33 g/L	*S. cerevisiae*	Dual compartmentalization (cytosol and peroxisome), enhancing peroxisomal supply of ATP and NADPH	Fed-batch	Zhang et al., 2024
	Valencene	Flavoring agent (fruity)	16.6 g/L	*S. cerevisiae*	Overexpression of key enzymes via the optimized GAL expression system	Fed-batch	Ye et al., 2022
	Nootkatone	Flavoring agent (fruity)	1.02 g/L	*S. cerevisiae*	Optimization HPO to AtCPR ratio	Fed-batch	Cha et al., 2022
	Nerolidol	Flavoring agent (woody and spicy)	497 mg/L	*S. cerevisiae*	GAL overexpression of the MVA pathway, reduction of competing pathways	Batch	Qu et al., 2020
Diterpenoid (C20)	Taxadiene	Nutraceutical precursor (paclitaxel)	528 mg/L	*S. cerevisiae*	Overexpression of key enzymes with high copy number, downregulation of squalene synthase	Batch	Karaca et al., 2024
	Sclareol	Sustainable precursor for ambergris	11.4 g/L	*S. cerevisiae*	Improvement of cytosolic acetyl-CoA and NADPH availabilities, deletion of targeted regulators	Fed-batch	Cao et al., 2023
Triterpenoid (C30)	Squalene	Antioxidant	32.8 g/L	*Y. lipolytica*	Peroxisomal compartmentalization, enhancement of peroxisomal acetyl-CoA supply	Fed-batch	Ma et al., 2024
	Amyrin	Antioxidant, emulsifier	2.6 g/L	*Y. lipolytica*	Partial peroxisomal compartmentalization, optimization of key enzyme expression levels	Fed-batch	Du et al., 2022

limonene production, most notably YALI0F19492p, whose overexpression resulted in an 8-fold increase in titer (Li et al., 2021).

β-Myrcene, another monoterpene prominent in hops and essential to beer's flavor profile, serves as a key flavoring agent in food and beverages while showing promise for therapeutic applications through its anti-inflammatory and antioxidant properties (Surendran et al., 2021; Zeng et al., 2023). Introduction of the dominant negative FPPS coupled with attenuated native FPPS expression effectively reduced metabolic flux toward FPP and squalene biosynthesis in engineered *S. cerevisiae* SQ14 (Xia et al., 2022; Zeng et al., 2023). This strategic modification preserved cell viability while redirecting carbon flux from sterol biosynthesis in the strain, which harbors enhanced mevalonate and β-oxidation pathways along with heterologous *Y. lipolytica* ATP-citrate lyase overexpression (Xia et al., 2022). The overexpression of the fusion protein of an exogenous β-myrcene synthase and the dominant negative FPPS led to 8.12 mg/L of β-myrcene titer (Zeng et al., 2023). Shu et al. achieved significant enhancement in β-myrcene production through comprehensive engineering of *S. cerevisiae*, implementing a multi-faceted strategy that involved β-myrcene synthase screening, peroxisomal compartmentalization, enzyme fusion with dominant negative FPPS, and targeted metabolic engineering to augment GPP availability. The resulting engineered strain demonstrated a β-myrcene titer of 142.64 mg/L through fed-batch fermentation (Shu et al., 2024).

Linalool, a monoterpenoid alcohol widely used as a flavoring agent, imparts floral and slightly spicy notes to culinary products. It also exhibits diverse therapeutic properties including anticancer, antimicrobial, neuroprotective, anxiolytic, and organ-protective effects (An et al., 2021). Zhou et al. demonstrated that a dual approach of protein and metabolic engineering optimizing precursor availability and enzyme efficiency effectively enhanced linalool biosynthesis in *S. cerevisiae*. Directed evolution generated a superior mutant of truncated limonene synthase from *Mentha citrata* (t67OMcLIS$^{E343D/E352H}$) that exhibits 52.7 % enhancement in linalool biosynthesis. Integration of the mutant limonene synthase and the dominant negative FPPS, combined with comprehensive overexpression of the MVA pathway in *S. cerevisiae*, achieved a linalool titer of 53.14 mg/L (Zhou et al., 2020). Linalool productivity of the engineered *S. cerevisiae* was further improved by attaching an N-terminal Ser-Lys-Ile-Lys tag to increase expression of the engineered linalool synthase, and implementing modular co-assembly of the linalool synthase and geranyl pyrophosphate synthase. Consequently, 80.9 mg/L of linalool titer in *S. cerevisiae* was achieved with additional downregulation of native farnesyl pyrophosphate synthase (Zhou et al., 2021). Another recent work on *S. cerevisiae* precision fermentation combined the dominant negative FPPS with *Actinidia arguta*-derived truncated linalool synthase, while introducing an isopentenol utilization pathway for optimized IPP/DMAPP production utilizing isoprenol and prenol supplement (Chatzivasileiou et al., 2019; Yaoyao Zhang et al., 2022). Through careful regulation of enzyme expression levels and downstream IPP flux, the engineered *S. cerevisiae* strain achieved a linalool titer of 142.88 mg/L in two-phase extractive fermentation (Yaoyao Zhang et al., 2022).

4.2. Sesquiterpenoids

Sesquiterpenoids represent the most diverse subgroup of terpenes in nature, encompassing several thousand distinct compounds that can form more than 300 different structural skeletons (Liu et al., 2022; Mai et al., 2021). In yeast cells, these compounds are derived from FPP, which also serves an essential role in the biosynthesis of sterols in yeasts. Thus, downregulating the expression of squalene synthase is a common metabolic engineering strategy for enhancing sesquiterpene production in yeasts (Fig. 2B) (Kwak et al., 2020; Liu et al., 2022).

α-Farnesene plays a significant role in apple aroma and finds applications in flavor formulations due to its distinctive fruity and floral notes (Huelin and Murray, 1966). Beyond its aromatic properties, this compound serves as a valuable precursor in the biosynthesis of essential nutrients, specifically vitamin E and vitamin K1 (Chen et al., 2023). In the context of precision fermentation, *K. phaffii* has emerged as a promising yeast platform for α-farnesene biosynthesis. Liu et al. validated a compartmentalization strategy that simultaneously utilized both cytosol and peroxisomes in *K. phaffii*; it incorporated the isopentenol utilization pathway as an efficient shortcut for IPP synthesis within peroxisomes, coupled with the heterologous expression of *E. coli* cytosolic pyruvate dehydrogenase to enhance acetyl-CoA availability in the cytosol. Through the synergistic combination of this dual-compartment regulation strategy and an optimized cofeeding regime utilizing oleic acid and sorbitol, the engineered *K. phaffii* strain achieved a remarkable α-farnesene titer of 2.56 ± 0.04 g/L (Liu et al., 2021). Cofactor engineering also represents a critical strategic approach for enhancing α-farnesene production in *K. phaffii*. The NADPH availability in *K. phaffii* was intensified through systematic engineering of key NADPH-dependent metabolic nodes, such as oxPPP (glucose 6-phosphate dehydrogenase, G6PD; 6-phosphogluconolactonase, 6PGL), NAD-dependent glycerol-3-phosphate dehydrogenase, and a heterologous NADH kinase (Chen et al., 2023). Together with elevating the cellular ATP pool through the overexpression of endogenous adenine phosphoribosyltransferase (Alfonzo et al., 1999), the comprehensive cofactor engineering approach culminated in an engineered *K. phaffii* strain capable of producing α-farnesene at titers of 3.09 ± 0.37 g/L (Chen et al., 2023).

α-Humulene, also known as α-caryophyllene, is another prominent sesquiterpene that has garnered substantial research attention for its production through yeast-based precision fermentation. It is distinguished by its characteristic aromatic properties and therapeutic potential, particularly its anti-inflammatory activity (Dalavaye et al., 2024; Mendes de Lacerda Leite et al., 2021). Rationale metabolic engineering for enhancing fluxes through the α-humulene synthetic route, including the MVA pathway, is the representative precision fermentation approach. As an example, engineered *C. tropicalis* overexpressing codon-optimized α-humulene synthase, FPPS, AACT, and NADH-dependent HMGR achieved an α-humulene titer of 4.12 g/L in fed-batch fermentation (Zhang et al., 2022). Subcellular compartmentalization has been adopted as a pivotal strategy in yeast precision fermentation for the enhanced production of α-humulene as well. *Y. lipolytica* was harnessed as a robust cellular platform for α-humulene biosynthesis through systematic peroxisomal engineering; the comprehensive approach integrated compartmentalization of the α-humulene pathway, enhancement of ATP and acetyl-CoA availability, and fine-tuning of rate-limiting enzymes through the optimization of their gene copy numbers. The resultant engineered *Y. lipolytica* strain achieved an α-humulene titer of 3.2 g/L in a 5 L bioreactor (Guo et al., 2021). On the other hand, another recent study enhanced the production of α-humulene in *S. cerevisiae* through dual-compartment engineering by constructing the α-humulene synthetic pathway in both cytosol and peroxisome (Zhang et al., 2024, 2020). The dual-compartment engineering resulted in a 2.5-fold increase in α-humulene production compared to strains engineered only in the cytoplasm. Through fed-batch fermentation, the engineered strain achieved an α-humulene titer of 1.73 mg/L (Zhang et al., 2020). The dual-compartment engineering strategy was further sophisticated using an orthogonal artificial peroxisome protein transport system, enabling more refined manipulation of target protein compartmentalization independent of the native trafficking system (Cross et al., 2017; Zhang et al., 2024). Additional metabolic optimizations, including the overexpression of peroxisomal adenine nucleotide transporter and isocitrate-2-oxo-glutarate redox shuttle, were performed to enhance the supply of ATP and NADPH in peroxisomes (Liu et al., 2020), achieving an α-humulene titer of 17.33 g/L through fed-batch fermentation (Zhang et al., 2024).

Valencene, a key constituent of essential oils from the *Citrus* genus, contributes significantly to the characteristic flavor and aroma of orange fruit. This sesquiterpene compound imparts woody citrus notes and serves as a valuable additive in food and beverages as well as fragrances

(Cao et al., 2022; Chen et al., 2019). Chen et al. demonstrated that valencene production in *S. cerevisiae* can be enhanced by increasing the FPP availability through downregulation of squalene synthase and deletion of the heme-dependent repressor of hypoxic genes in the MVA pathway (Rox1p). Additionally, metabolic fluxes toward FPP were maximized via additional overexpression of all upstream MVA pathway genes. After optimizing the expression level of valencene synthase from *Callitropsis nootkatensis* through promoter and terminator engineering, the engineered *S. cerevisiae* achieved a valencene titer of 539.3 mg/L in fed-batch fermentation (Chen et al., 2019). The MVA pathway engineering approach substantially enhanced valencene production in a metabolically reprogrammed *S. cerevisiae* strain that featured rewired central carbon metabolism optimized for lipogenesis (Yu et al., 2018); after the optimization of the number of *C. nootkatensis* valencene synthase expression cassettes, the engineered strain produced 1.2 g/L of valencene in fed-batch fermentation (Cao et al., 2022). The yeast precision fermentation system for valencene production could be advanced further through the screening for valencene synthase variants with higher enzymatic activity. For instance, a recent study screened a valencene synthase from *Eryngium glaciale*, exhibiting better catalytic activity than the conventional enzyme from *C. nootkatensis*, and further improved its activity through site-directed mutagenesis. In addition, expression levels of the mutant *E. glaciale* valencene synthase, truncated HMGR, and FPPS were enhanced under the control of GAL promoters in the *GAL80* knockout *S. cerevisiae* strain (Ye et al., 2022). Additionally, the GAL induction was coupled with the production of Ura3p, which is critical to the growth of the corresponding auxotrophic *ura3Δ S. cerevisiae*, to maintain normal operation of this GAL expression system (Shi et al., 2019; Ye et al., 2022). Fed-batch fermentation of the resultant *S. cerevisiae* strain recorded a valencene titer of 16.6 g/L (Ye et al., 2022).

Nootkatone represents a significant sesquiterpene ketone compound characterized by its potent organoleptic properties, particularly its distinctive grapefruit aroma and flavor profile. This compound plays a crucial role in the food and beverage industries as a natural flavor enhancer, where it is employed to impart and intensify citrus notes (Cha et al., 2022; Mai et al., 2021). The biosynthetic pathway for nootkatone production involves the enzymatic oxidation of valencene, proceeding through β-nootkatol as a key intermediate metabolite. For the initial oxidation step of valencene to β-nootkatol, two key enzymes, *Hyoscyamus muticus* premnaspirodiene oxygenase (HPO) and *Arabidopsis thaliana* cytochrome P450 reductase (AtCPR), have been optimized and incorporated into engineered *S. cerevisiae* strains carrying optimized valencene synthetic pathways; in this system, HPO catalyzes the hydroxylation of valencene to nootkatol, while AtCPR functions as its essential redox partner (Cha et al., 2022; Meng et al., 2020). The subsequent introduction of a short-chain dehydrogenase/reductase superfamily dehydrogenase from *Zingiber zerumbet* enabled the engineered strain to produce 59.78 mg/L of nootkatone by converting β-nootkatol to nootkatone in the engineered *S. cerevisiae* (Meng et al., 2020). Another recent work demonstrated that optimizing the expression ratio of HPO to AtCPR significantly elevated valencene oxidation in *S. cerevisiae*. By incorporating additional copies of the endogenous alcohol dehydrogenase gene, the engineered strain achieved a nootkatone titer of 1.02 g/L in fed-batch fermentation (Cha et al., 2022).

Nerolidol is a sesquiterpene alcohol with floral and woody notes found naturally in ginger and lemongrass and widely used as a flavoring agent in the food industry (Chan et al., 2016). Its potential antimicrobial, anti-inflammatory, and antioxidant properties have also been demonstrated (Chan et al., 2016; Raj et al., 2023). In a study focused on enhancing nerolidol production in *S. cerevisiae*, metabolic engineering strategies included increasing metabolic flux through the MVA pathway and reducing activities of competing pathways toward squalene and GGPP. Target genes were expressed under the control of GAL promoters, while additional overexpression of the transcription factor Hac1p enhanced GAL promoter stability, ultimately achieving a nerolidol titer of 497 mg/L with improved cell vitality (Qu et al., 2020).

4.3. Diterpenoids

The yeast-based precision fermentation for diterpenoid biosynthesis presents distinct challenges compared to smaller terpenes. The challenges mainly stem from more intricate molecular architectures and the requirement of an extra IPP unit to convert FPP into GGPP, the essential precursor for all diterpene scaffolds. These metabolic and structural complexities create substantial bottlenecks in developing robust engineered yeast strains as production platforms for diterpenes (Bureau et al., 2023; Liu et al., 2022). As GGPP is synthesized from FPP, downregulation of squalene synthase effectively redirects metabolic flux from sterol biosynthesis toward desired diterpenes, similar to common strategies used in sesquiterpene production (Fig. 2B) (Karaca et al., 2024).

Taxadiene is one of the representative diterpene molecules that can be produced as a key intermediate for the biological synthesis of paclitaxel, an FDA-approved natural compound efficacious against diverse diseases including ovarian cancer and Kaposi's sarcoma (Karaca et al., 2024; Nowrouzi et al., 2020). The enzymatic conversion of GGPP to taxadiene by taxadiene synthase is the primary rate-limiting step in the taxadiene biosynthetic pathway within engineered yeasts. To overcome this bottleneck, a synthetic fusion construct combining three key elements was devised (Nowrouzi et al., 2020); an N-terminal maltose-binding protein was combined with a codon-optimized taxadiene synthase as a solubility-enhancing tag, and $ERG20^{F96C}$, the engineered FPPS generating GGPP (Ignea et al., 2015), was additionally included for better substrate supply via colocalization. Multi-copy chromosomal integration of this construct in *S. cerevisiae* led to a 22-fold enhancement in taxadiene production compared to control strains. Through the synergistic application of synthetic biology tools for protein engineering and optimized bioprocess conditions, the engineered strain achieved taxadiene titers of 129 mg/L in shake flasks and 127 mg/L in a bioreactor (Nowrouzi et al., 2020). Differential subcellular compartmentalization of key heterogeneous enzymes is also a potential challenge in optimizing taxadiene biosynthesis in *S. cerevisiae*. This was evidenced in a terpene synthetic pathway incorporating GGPPS from *Sulfolobus acidocaldarius* and taxadiene synthase from *Taxus brevifolia*, which generated a relatively modest taxadiene titer of 10.2 mg/L while accumulating substantial amounts of the byproduct geranylgeraniol (214.2 mg/L) in *S. cerevisiae*. This inefficient production was primarily attributed to the mitochondrial localization of the taxadiene synthase. Fusion protein strategy combining the heterologous GGPPS with an engineered taxadiene synthase variant featuring optimized N-terminus truncation resulted in a 54 % improvement in taxadiene yield. Further optimization through increased copy number integration of the fusion protein expression cassette, coupled with fed-batch fermentation strategies, ultimately achieved taxadiene titers of 184.2 mg/L in the engineered *S. cerevisiae* (Zhang et al., 2023).

Optimization of native metabolism in host cells is equally critical as optimizing the heterologous taxadiene synthetic pathway. Karaca et al. demonstrated that overexpression of the whole MVA pathway including truncated HMGR and downregulated expression of squalene synthase enhanced farnesyl pyrophosphate availability considerably in *S. cerevisiae*. Additional episomal overexpression of truncated HMGR, FPPS, GGPPS, and taxadiene synthase in the resulting engineered strain achieved a substantial taxadiene titer of 528 mg/L (Karaca et al., 2024). The application of in silico design algorithms to yeast genome-scale metabolic models is a powerful approach for rational identification of engineering targets to enhance biosynthesis of terpene compounds, including taxadiene. Specifically, constraint-based reconstruction and analysis (Heirendt et al., 2019) successfully prioritized four high-impact genetic modifications in *S. cerevisiae*, namely *ILV2* (acetolactate synthase), *TRR1* (thioredoxin reductase), *ADE13* (adenylosuccinate lyase), and *ECM31* (3-methyl-2-oxobutanoate hydroxymethyltransferase). Implementation of these computationally predicted modifications in the

yeast host resulted in a 50 % enhancement in taxadiene production, achieving a titer of 215 mg/L, thereby validating the predictive power of genome-scale metabolic modeling for systematic strain optimization (Malcı et al., 2023).

Sclareol is another valuable diterpenoid compound with applications in the food and cosmetics industries. This bicyclic diterpene alcohol has garnered significant industrial interest as a sustainable precursor for ambergris-like fragrances, traditionally sourced from sperm whale intestinal secretions (Zhou et al., 2022). *S. cerevisiae* has been considered an efficient host platform for precision fermentation, circumventing the costly and labor-intensive traditional extraction process for sclareol production. To optimize cellular metabolism for sclareol overproduction, systematic rewiring of central metabolism, the MVA pathway, and corresponding regulatory factors has been implemented. Enhanced expression of MVA pathway core elements, including AACT, truncated HMGR, GGPP-generating mutant FPPS ($ERG20^{F96C}$), and NADH-dependent HMGR (Ignea et al., 2015; Meadows et al., 2016), elevated sclareol production in engineered *S. cerevisiae*. The integration of MVA pathway engineering with improved substrate and cofactor supply—specifically cytosolic acetyl-CoA and NADPH—through attenuation of fatty acid synthesis synergistically enhanced sclareol titers. Further optimization through additional MVA pathway overexpression, GGPPS fusion protein implementation, and deletion of targeted regulator-coding genes (*ROX1, DOS2, VBA5, YER134C, YNR063W*, and *YGR259C*) achieved sclareol production of 11.4 g/L in fed-batch fermentation (Cao et al., 2023). A recent study examining cofactor engineering strategies for enhanced NADPH supply and improved sclareol production demonstrated that G6PD, which catalyzes the initial conversion in the oxPPP, represents the optimal engineering target in *S. cerevisiae*. The implementation of fusion proteins combining G6PD with 6PGL, coupled with rational mutagenesis of G6PD (N403D and S238Q/I239F), significantly enhanced sclareol production in *S. cerevisiae*. (Adusumilli et al., 2024)

4.4. Triterpenoids

Triterpenoids are widely valued for applications in food, pharmaceuticals, and cosmetics due to their bioactive properties. In yeasts, triterpenoids can be synthesized from squalene, a native and essential triterpene that serves as a pivotal intermediate in the biosynthesis of ergosterol, the primary sterol component of yeast cell membranes (Dinday and Ghosh, 2023; Jordá and Puig, 2020). The biosynthetic pathway of triterpenoids predominantly initiates with the epoxidation of squalene to 2,3-oxidosqualene, which subsequently undergoes enzymatic cyclization to generate diverse triterpene scaffolds. These basic skeletal structures are further subject to various post-cyclization modifications, including oxidative transformations, acyl group additions, and glycosidic conjugations, yielding an extensive array of structurally complex triterpenoid derivatives (Guo et al., 2020). Recent investigation characterized squalene-independent triterpene biosynthetic pathways from fungal species (Tao et al., 2022), but these pathways have yet to be exploited for yeast metabolic engineering applications for triterpenoid production.

Squalene, beyond its fundamental role as a biosynthetic precursor in triterpenoid metabolism, represents a commercially significant triterpenoid compound with extensive applications across multiple industrial sectors. The integration of squalene into functional food matrices has garnered considerable attention due to its multifaceted physiological effects, including its potent antioxidative properties and its capacity to modulate glycoprotein homeostasis in plasma and cardiac tissues (Du et al., 2023; Micera et al., 2020). The accumulation of squalene occurs inherently in *S. cerevisiae* when its biosynthetic pathway is upregulated, attributed to the stringent regulatory mechanisms governing post-squalene ergosterol biosynthesis (Jordá and Puig, 2020; Mantzouridou and Tsimidou, 2010). Therefore, metabolic engineering strategies to enhance squalene biosynthesis in yeasts primarily focused on optimizing the MVA pathway flux and NADPH availability. For instance, overexpression of key enzymes, particularly truncated HMGR, FPPS, isopentenyl diphosphate:dimethylallyl diphosphate isomerase (IDI), and squalene synthase, significantly elevated squalene accumulation in *S. cerevisiae* (Li et al., 2020). The introduction of heterologous NADH-dependent HMGR (Meadows et al., 2016), coupled with enhanced ethanol utilization through overexpression of ethanol-oxidizing alcohol dehydrogenase and heterologous acetaldehyde dehydrogenase, resulted in substantial squalene production (9.472 g/L) in fed batch fermentation, demonstrating industrial viability (Li et al., 2020). Lu et al. demonstrated that concurrent overexpression of NADPH-dependent HMGR and NADP-dependent glyceraldehyde 3-phosphate dehydrogenase presents an alternative to NADH-dependent HMGR for enhancing squalene biosynthesis via the MVA pathway. Their findings also revealed a significant correlation between β-alanine metabolism and elevated MVA pathway activity in *S. cerevisiae*. Through optimizing NADPH availability, β-alanine metabolism, and cytosolic acetyl-CoA supply via pyruvate kinase-decarboxylase fusion, they achieved a squalene titer of 4.94 g/L in fed-batch fermentation using cane molasses medium (Lu et al., 2022). On the other hand, Xia et al. showed the potential of random mutagenesis to enhance squalene production in *S. cerevisiae*. They first fortified both the MVA pathway and β-oxidation pathway through the multi-copy integration of genes encoding truncated HMGR, IDI, and ATP-citrate lyase from *Y. lipolytica* (Xia et al., 2022). They subsequently performed random mutagenesis through atmospheric and room-temperature plasma mutagenesis (Li et al., 2024) and identified superior squalene-producing *S. cerevisiae* strains via high-throughput screening using Nile red staining. The best screened strain achieved 8.2 g/L of squalene titer by fed-batch fermentation in a 5 L bioreactor (Xia et al., 2022).

As an oleaginous yeast, *Y. lipolytica* exhibits distinct advantages in the production and intracellular accumulation of hydrophobic terpenoids, which present significant challenges in cellular export, such as squalene (Zhang et al., 2023). An engineered *Y. lipolytica* strain, simply overexpressing homologous HMGR and diacylglycerol acyltransferase, which synthesizes triacylglycerol and consequently promotes lipid droplet formation in the cell, achieved a squalene titer of 731.18 mg/L in an optimized medium with acetate, citrate, and terbinafine additives (Tang et al., 2021). concurrent overexpression of the oxPPP, *S. cerevisiae*-derived HMGR, and endogenous squalene synthase. Further metabolic optimization via integration of an isoprenol utilization pathway and upregulation of diacylglycerol acyltransferase culminated in a squalene titer of 1628.2 mg/L with an isoprenol additive (Liu et al., 2024b). To achieve industrial squalene production in *Y. lipolytica*, it was imperative to circumvent constraints imposed by competing pathways and precursor availability, particularly in cytosolic sterol biosynthesis. Notably, compartmentalization of the complete squalene biosynthetic pathway in peroxisomes effectively segregated the process from competing cytosolic reactions. Enhancement of peroxisomal acetyl-CoA supply through dual strategies—lipid conversion and establishment of a CO_2-derived acetate pathway—substantially augmented squalene accumulation. The engineered strains demonstrated remarkable production capacity, achieving squalene titers of 32.8 g/L and 31.6 g/L from glucose and acetate substrates, respectively, in fed-batch fermentation (Ma et al., 2024).

Amyrin, a pentacyclic triterpene, demonstrates significant utility through its antioxidant properties that enable natural preservation, while also enhancing flavor profiles in specific food formulations and functioning as a natural emulsifier in food processing applications (Santos et al., 2012; Su et al., 2024). In the realm of functional foods, amyrin also has gained considerable attention for its anti-inflammatory activity (Simão da Silva et al., 2011), making it an increasingly valuable ingredient in the development of health-promoting food products. Amyrin production in yeast hosts requires heterologous expression of amyrin synthase, as it is not a native metabolite. Recent studies demonstrated successful amyrin biosynthesis in *Y. lipolytica* through

heterologous expression of multi-functional amyrin synthase from *Catharanthus roseus*. Metabolic flux toward amyrin precursor, namely 2, 3-oxidosqualene, was also enhanced through overexpression of key enzymes of its biosynthetic pathway (HMGR, FPPS, squalene synthase, squalene epoxidase). Integration of semi-rational amyrin synthase engineering with enhanced precursor supply achieved more than 20-fold increase in amyrin production. Under optimized fermentation conditions, the engineered *Y. lipolytica* strain produced approximately 120 mg/L total amyrins (Kong et al., 2022). Peroxisome compartmentalization represents another strategic approach for enhancing β-amyrin production in yeasts by mitigating the inhibitory effects of over-accumulated squalene on 2,3-oxidosqualene cyclases, including amyrin synthase (Duriatti et al., 1985; Taton et al., 1992). Indeed, implementation of peroxisomal compartmentalization of the squalene synthetic pathway yielded a 2.6-fold increase in amyrin titer compared to cytosolic engineering with amyrin synthase from *Glycyrrhiza glabra*. Further optimization of squalene epoxidase and amyrin synthase expression, coupled with lanosterol synthase attenuation, achieved amyrin titers of 2.6 g/L in fed-batch fermentation (Du et al., 2022).

5. Expanding the range of producible terpene and terpenoid compounds

Despite the extensive structural diversity of terpene molecules, the identification and cataloging of terpene biosynthetic elements still remain disproportionately limited (Bian et al., 2017). Sequence similarity-based prediction approaches have traditionally facilitated the screening of potential terpene synthase genes (Chen et al., 2021; Liang et al., 2023). However, their utility in identifying bacterial terpene synthases has been limited by the notably low sequence conservation between prokaryotic and eukaryotic terpene synthases, despite the predominance of plant terpene synthases in current databases (Rudolf and Chang, 2020; Yamada et al., 2015). Functional metagenomic screening has emerged as an alternative strategy, harnessing the toxicity of overproduced prenyl pyrophosphate in *E. coli*, to identify novel terpene synthases from metagenomic specimens (Kwak et al., 2022; Martin et al., 2003). Through the implementation of an engineered *E. coli* strain with minimized mutation rate and optimized screening parameters, this approach has demonstrated applicability in identifying novel terpene synthase candidates. A notable example is the successful isolation of a novel β-farnesene synthase from human fecal metagenomes, which exhibited no sequence homology to known β-farnesene synthases and achieved a β-farnesene titer of 120 mg/L when expressed in engineered *S. cerevisiae* (Kwak et al., 2022). This sequence-independent screening presents a promising avenue for expanding the diversity of characterized terpene synthases by accessing the vast enzymatic potential within uncultivated microbial communities, thereby enhancing the spectrum of producible terpene compounds through precision fermentation.

Still, biosynthetic potentials and subsequent application scopes of terpene molecules are fundamentally constrained by the limited diversity of natural building blocks. Recent advances in synthetic biology and protein engineering strategically expanded the range of producible terpene molecules in yeast via synthetic pathways for noncanonical C16 building blocks and identified compatible elements for terpene biosynthesis. The resultant platform overcame innate building block limitations and produced 28 noncanonical terpenes, including novel terpene compounds with promising odorant properties, suggesting potential applications in the flavor and fragrance industries (Ignea et al., 2022). This result not only validated the feasibility of expanding the range of producible terpenes but also establishes a systematic framework for producing structurally diverse terpenes. The modular nature of this platform, combined with its capacity for producing novel compounds with desirable properties, presents significant opportunities for expanding the repertoire of sustainable bioproduction systems for specialized flavoring substances.

6. Addressing genetic and metabolic instabilities during yeast-based terpenoid production

Despite multiple advantages of yeast hosts over prokaryotes, the genetic and metabolic instabilities of engineered yeast strains may still cause a critical bottleneck in industrial terpenoid production by compromising long-term fermentation performance. Metabolic burden caused by the overexpression of additional metabolic pathways, and the potential toxicity associated with the accumulation of intermediates and end products, are the major factors triggering the instabilities (Caunt et al., 1988; Mason, 1991). These instabilities encompass interconnected phenomena. Specifically, loss of terpenoid biosynthetic genes from multi-copy plasmids over successive generations and metabolic perturbation resulting from the pathway imbalances can generate toxic intermediates, disrupt cellular homeostasis, or inhibit the activity of key enzymes through feedback regulation mediated by intermediate accumulation (Bureau et al., 2023; Chen et al., 2023). A recent study demonstrated that even non-natural substrates can severely impact cellular physiology; specifically, isopentenol inhibits energy metabolism, leading to reduced efficiency of the isopentenol utilization pathway in *S. cerevisiae* (Li et al., 2024). Comprehensive engineering approaches that integrate stable genomic integration strategies, advanced process control systems, and systems biology-guided strain optimization are being actively developed to address these challenges (Bureau et al., 2023; Chen et al., 2023; Rugbjerg and Sommer, 2019). For instance, genomic integration demonstrates significant advantages over plasmid-based expression systems, eliminating selective pressure requirements during fermentation while providing enhanced genetic stability compared to episomal vectors, thereby achieving substantial improvements in monoterpene, sesquiterpene, and triterpene titers (Da Silva and Srikrishnan, 2012; Mukherjee et al., 2022). Concurrently, machine learning-guided pathway optimization employing combinatorial strain libraries coupled with high-throughput screening methodologies synergistically amplifies production outcomes when integrated with chromosomal expression strategies (Mukherjee et al., 2022).

7. Challenges and future perspectives

While significant advances in synthetic biology have demonstrated the technical feasibility of producing diverse terpenoids across multiple yeast platforms, several key challenges must be addressed to achieve widespread commercial implementation in food applications. The most considerable unresolved challenge lies in bridging the gap between laboratory-scale achievements and industrial viability, which requires genetic stability, process economics, and regulatory compliance simultaneously (Meadows et al., 2016; Walls and Rios-Solis, 2020). Scale-up of microbial processes requires a high-stakes financial investment typically exceeding that required for process development in the laboratory, with scale factors ranging from thousands to millions. The fundamental challenge stems from the non-linear nature of bioprocess scale-up, where conditions optimized at laboratory scale are often suboptimal at larger scales, and slight deviations can be extraordinarily costly.

Regarding the selection of a yeast platform for food-grade terpenoid production, *S. cerevisiae* remains the most promising near-term option due to its established species-level GRAS status, which enables flexible strain engineering without requiring individual regulatory approval for each genetic modification. Still, its Crabtree-positive metabolism fundamentally limits efficiency for energy-intensive terpenoid biosynthesis, necessitating complicated metabolic and environmental perturbations to circumvent glucose repression. Although multiple studies have demonstrated that the utilization of non-fermentable sugars, particularly xylose, is a promising strategy for enhancing terpenoid biosynthesis in *S. cerevisiae* by circumventing the Crabtree effect (Kwak et al., 2019), lignocellulosic biomass inherently contains both xylose and glucose, necessitating sophisticated fermentation strategies for effective glucose-xylose co-utilization (Kim et al., 2012; Zhang et al., 2015). In

practice, current industrial applications of lignocellulosic biomass-based *S. cerevisiae* bioprocesses remain predominantly limited to the production of relatively simpler metabolites, such as ethanol (Chandel et al., 2021; Lynd et al., 2017; Padella et al., 2019). On the other hand, *K. phaffii* and *Y. lipolytica*, despite their superior metabolic characteristics for terpenoid production as oleaginous yeasts, face regulatory barriers due to strain-specific GRAS requirements. Although the barriers potentially limit their commercial viability for food applications, the development of novel GRAS-approved oleaginous yeast strains represents a critical research priority that could transform the landscape of industrial terpenoid production. Addressing these platform-specific limitations will require strategic integration of emerging technologies and interdisciplinary approaches, such as synthetic biology tools with systems-level understanding of cellular metabolism (Bureau et al., 2023). The convergence of these technological advances with evolving regulatory frameworks and increasing market demand for sustainable ingredients suggests that yeast-based terpenoid production will play an increasingly important role in the future bioeconomy, though successful implementation will require continued interdisciplinary collaboration including metabolic engineering, bioprocess engineering, and regulatory science.

8. Conclusion

Yeast-based precision fermentation has demonstrated remarkable potential for sustainable food-grade production of terpenoids, offering solutions to the limitations inherent in traditional plant-based extraction methods. For food-grade terpenoid production, *S. cerevisiae* emerges as the most promising platform due to its established species-level GRAS status, extensive regulatory acceptance, extensive genetic tools, and proven industrial scalability. However, Y. lipolytica presents compelling advantages for specific applications. Recent advances in metabolic engineering strategies—particularly the synergistic implementation of pathway optimization, compartmentalization, and cofactor engineering—have achieved unprecedented production metrics across various terpenoid classes. The emergence of innovative strategies, such as orthogonal pathway development and artificial compartmentalization systems, has further expanded the capabilities of yeast platforms. The integration of functional metagenomic screening with advanced protein engineering approaches offers promising avenues for discovering novel terpene synthases and expanding the range of producible terpenoid compounds. The development of synthetic pathways for noncanonical building blocks represents another frontier, potentially enabling the biosynthesis of novel terpenoid structures with unique properties. Additionally, as production scales increase, addressing challenges related to product toxicity, metabolic burden, and process economics will be crucial. The continuous evolution of synthetic biology tools, coupled with a deeper understanding of cellular metabolism, will position yeast-based precision fermentation as an increasingly robust platform for sustainable terpenoid production in food and nutraceutical applications.

CRediT authorship contribution statement

Juhan Pak: Writing – original draft, Investigation, Conceptualization. **Hyeongwoo Park:** Writing – original draft, Investigation. **Kangmin Baek:** Writing – review & editing, Investigation. **Hong-Seok Son:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Suryang Kwak:** Writing – original draft, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this papeThis section comprises references that occur in the reference list but not in the body of the text. Please position each reference in the text or, alternatively, delete it. Any reference not dealt with will be retained in this section. Thank you.r.

Funding sources

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (RS-2023–00253771 and RS-2025–00522182), Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (RS-2024–00402850), the Biomaterials Specialized Graduate Program through the Korea Environmental Industry & Technology Institute (KEITI) funded by the Ministry of Environment (MOE), and a Korea University Grant, and the Institute of Biomedical Science & Food Safety, CJ Food Safety Hall at Korea University, Republic of Korea.

Data availability

No data was used for the research described in the article.

References

Adusumilli, S.H., Alikkam Veetil, A., Choudhury, C., Chattopadhyaya, B., Behera, D., Bachhawat, A.K., 2024. Glucose 6-phosphate dehydrogenase variants increase NADPH pools for yeast isoprenoid production. FEBS Open Bio. 14, 410–425. https://doi.org/10.1002/2211-5463.13755.

Ajikumar, P.K., Xiao, W.-H., Tyo, K.E.J., Wang, Y., Simeon, F., Leonard, E., Mucha, O., Phon, T.H., Pfeifer, B., Stephanopoulos, G., 2010. Isoprenoid pathway optimization for Taxol precursor overproduction in *Escherichia coli*. Science 330, 70–74. https://doi.org/10.1126/science.1191652.

Alfonzo, J.D., Crother, T.R., Guetsova, M.L., Daignan-Fornier, B., Taylor, M.W., 1999. *APT1*, but not *APT2*, codes for a functional adenine phosphoribosyltransferase in *Saccharomyces cerevisiae*. J. Bacteriol. 181, 347–352. https://doi.org/10.1128/JB.181.1.347-352.1999.

An, Q., Ren, J.-N., Li, X., Fan, G., Qu, S.-S., Song, Y., Li, Y., Pan, S.-Y., 2021. Recent updates on bioactive properties of linalool. Food Funct. 12, 10370–10389. https://doi.org/10.1039/d1fo02120f.

Arendt, P., Miettinen, K., Pollier, J., De Rycke, R., Callewaert, N., Goossens, A., 2017. An endoplasmic reticulum-engineered yeast platform for overproduction of triterpenoids. Metab. Eng. 40, 165–175. https://doi.org/10.1016/j.ymben.2017.02.007.

Arnesen, J.A., Kildegaard, K.R., Cernuda Pastor, M., Jayachandran, S., Kristensen, M., Borodina, I., 2020. *Yarrowia lipolytica* strains engineered for the production of terpenoids. Front. Bioeng. Biotechnol. 8, 945. https://doi.org/10.3389/fbioe.2020.00945.

Baumann, K., Carnicer, M., Dragosits, M., Graf, A.B., Stadlmann, J., Jouhten, P., Maaheimo, H., Gasser, B., Albiol, J., Mattanovich, D., Ferrer, P., 2010. A multi-level study of recombinant *Pichia pastoris* in different oxygen conditions. BMC Syst. Biol. 4, 141. https://doi.org/10.1186/1752-0509-4-141.

Bian, G., Deng, Z., Liu, T., 2017. Strategies for terpenoid overproduction and new terpenoid discovery. Curr. Opin. Biotechnol. 48, 234–241. https://doi.org/10.1016/j.copbio.2017.07.002.

Bolzico, B.C., Racca, S., Khawam, J.N., Leonardi, R.J., Tomassi, A.H., Benzzo, M.T., Comelli, R.N., 2024. Exploring xylose metabolism in non-conventional yeasts: kinetic characterization and product accumulation under different aeration conditions. J. Ind. Microbiol. Biotechnol. 51, kuae023. https://doi.org/10.1093/jimb/kuae023.

Bureau, J.-A., Oliva, M.E., Dong, Y., Ignea, C., 2023. Engineering yeast for the production of plant terpenoids using synthetic biology approaches. Nat. Prod. Rep. 40, 1822–1848. https://doi.org/10.1039/d3np00005b.

Câmara, J.S., Perestrelo, R., Ferreira, R., Berenguer, C.V., Pereira, J.A.M., Castilho, P.C., 2024. Plant-derived terpenoids: a plethora of bioactive compounds with several health functions and industrial applications-a comprehensive overview. Molecules 29, 3861. https://doi.org/10.3390/molecules29163861.

Cao, C., Cao, X., Yu, W., Chen, Y., Lin, X., Zhu, B., Zhou, Y.J., 2022. Global metabolic rewiring of yeast enables overproduction of sesquiterpene (+)-valencene. J. Agric. Food. Chem. 70, 7180–7187. https://doi.org/10.1021/acs.jafc.2c01394.

Cao, X., Yu, W., Chen, Y., Yang, S., Zhao, Z.K., Nielsen, J., Luan, H., Zhou, Y.J., 2023. Engineering yeast for high-level production of diterpenoid sclareol. Metab. Eng. 75, 19–28. https://doi.org/10.1016/j.ymben.2022.11.002.

Caputi, L., Aprea, E., 2011. Use of terpenoids as natural flavouring compounds in food industry. Recent. Pat. Food Nutr. Agric. 3, 9–16. https://doi.org/10.2174/2212798411103010009.

Carsanba, E., Pintado, M., Oliveira, C., 2021. Fermentation strategies for production of pharmaceutical terpenoids in engineered yeast. Pharmaceuticals 14, 295. https://doi.org/10.3390/ph14040295.

Caunt, P., Impoolsup, A., Greenfield, P.F., 1988. Stability of recombinant plasmids in yeast. J. Biotechnol. 8, 173–192. https://doi.org/10.1016/0168-1656(88)90001-6.
Cha, Y., Li, W., Wu, T., You, X., Chen, H., Zhu, C., Zhuo, M., Chen, B., Li, S., 2022. Probing the synergistic ratio of P450/CPR to improve (+)-nootkatone production in *Saccharomyces cerevisiae*. J. Agric. Food Chem. 70, 815–825. https://doi.org/10.1021/acs.jafc.1c07035.
Chan, W.-K., Tan, L.T.-H., Chan, K.-G., Lee, L.-H., Goh, B.-H., 2016. Nerolidol: a sesquiterpene alcohol with multi-faceted pharmacological and biological activities. Molecules 21, 529. https://doi.org/10.3390/molecules21050529.
Chandel, A.K., Forte, M.B.S., Gonçalves, I.S., Milessi, T.S., Arruda, P.V., Carvalho, W., Mussatto, S.I., 2021. Brazilian biorefineries from second generation biomass: critical insights from industry and future perspectives. Biofuels. Bioprod. Biorefin. 15, 1190–1208. https://doi.org/10.1002/bbb.2234.
Chang, M.C.Y., Keasling, J.D., 2006. Production of isoprenoid pharmaceuticals by engineered microbes. Nat. Chem. Biol. 2, 674–681. https://doi.org/10.1038/nchembio836.
Chatzivasileiou, A.O., Ward, V., Edgar, S.M., Stephanopoulos, G., 2019. Two-step pathway for isoprenoid synthesis. Proc. Natl. Acad. Sci. U.S.A. 116, 506–511. https://doi.org/10.1073/pnas.1812935116.
Chen, H., Zhu, C., Zhu, M., Xiong, J., Ma, H., Zhuo, M., Li, S., 2019. High production of valencene in *Saccharomyces cerevisiae* through metabolic engineering. Microb. Cell Fact. 18, 195. https://doi.org/10.1186/s12934-019-1246-2.
Chen, L., Xiao, W., Yao, M., Wang, Y., Yuan, Y., 2023a. Compartmentalization engineering of yeasts to overcome precursor limitations and cytotoxicity in terpenoid production. Front. Bioeng. Biotechnol. 11, 1132244. https://doi.org/10.3389/fbioe.2023.1132244.
Chen, S.-L., Liu, T.-S., Zhang, W.-G., Xu, J.-Z., 2023b. Cofactor engineering for efficient production of α-farnesene by rational modification of NADPH and ATP regeneration pathway in *Pichia pastoris*. Int. J. Mol. Sci. 24, 1767. https://doi.org/10.3390/ijms24021767.
Chen, Z., Vining, K.J., Qi, X., Yu, X., Zheng, Y., Liu, Z., Fang, H., Li, L., Bai, Y., Liang, C., Li, W., Lange, B.M., 2021. Genome-wide analysis of terpene synthase gene family in *Mentha longifolia* and catalytic activity analysis of a single terpene synthase. Genes (Basel) 12, 518. https://doi.org/10.3390/genes12040518.
Chou, A., Lee, S.H., Zhu, F., Clomburg, J.M., Gonzalez, R., 2021. An orthogonal metabolic framework for one-carbon utilization. Nat. Metab. 3, 1385–1399. https://doi.org/10.1038/s42255-021-00453-0.
Cross, L.L., Paudyal, R., Kamisugi, Y., Berry, A., Cuming, A.C., Baker, A., Warriner, S.L., 2017. Towards designer organelles by subverting the peroxisomal import pathway. Nat. Commun. 8, 454. https://doi.org/10.1038/s41467-017-00487-7.
Da Silva, N.A., Srikrishnan, S., 2012. Introduction and expression of genes for metabolic engineering applications in *Saccharomyces cerevisiae*. FEMS Yeast Res. 12, 197–214. https://doi.org/10.1111/j.1567-1364.2011.00769.x.
Dai, Z., Huang, M., Chen, Y., Siewers, V., Nielsen, J., 2018. Global rewiring of cellular metabolism renders *Saccharomyces cerevisiae* Crabtree negative. Nat. Commun. 9, 3059. https://doi.org/10.1038/s41467-018-05409-9.
Dalavaye, N., Nicholas, M., Pillai, M., Erridge, S., Sodergren, M.H., 2024. The clinical translation of α-humulene - a scoping review. Planta Med. 90, 664–674. https://doi.org/10.1055/a-2307-8183.
Dinday, S., Ghosh, S., 2023. Recent advances in triterpenoid pathway elucidation and engineering. Biotechnol. Adv. 68, 108214. https://doi.org/10.1016/j.biotechadv.2023.108214.
Du, M.-M., Zhu, Z.-T., Zhang, G.-G., Zhao, Y.-Q., Gao, B., Tao, X.-Y., Liu, M., Ren, Y.-H., Wang, F.-Q., Wei, D.-Z., 2022. Engineering *Saccharomyces cerevisiae* for hyperproduction of β-amyrin by mitigating the inhibition effect of squalene on β-amyrin synthase. J. Agric. Food. Chem. 70, 229–237. https://doi.org/10.1021/acs.jafc.1c06712.
Du, X., Ma, X., Gao, Y., 2023. The physiological function of squalene and its application prospects in animal husbandry. Front. Vet. Sci. 10, 1284500. https://doi.org/10.3389/fvets.2023.1284500.
Duriatti, A., Bouvier-Nave, P., Benveniste, P., Schuber, F., Delprino, L., Balliano, G., Cattel, L., 1985. In vitro inhibition of animal and higher plants 2,3-oxidosqualene-sterol cyclases by 2-aza-2,3-dihydrosqualene and derivatives, and by other ammonium-containing molecules. Biochem. Pharmacol. 34, 2765–2777. https://doi.org/10.1016/0006-2952(85)90578-7.
Eddin, L.B., Jha, N.K., Meeran, M.F.N., Kesari, K.K., Beiram, R., Ojha, S., 2021. Neuroprotective potential of limonene and limonene containing natural products. Molecules 26, 4535. https://doi.org/10.3390/molecules26154535.
EFSA Panel on Food Additives and Flavourings (FAF), Younes, M., Aquilina, G., Degen, G., Engel, K.-H., Fowler, P., Frutos Fernandez, M.J., Fürst, P., Gundert-Remy, U., Gürtler, R., Husøy, T., Manco, M., Mennes, W., Passamonti, S., Moldeus, P., Shah, R., Waalkens-Berendsen, I., Wright, M., Barat Baviera, J.M., Gott, D., Herman, L., Leblanc, J.-C., Wölfle, D., Entrena, J.A., Consuelo, C., Mech, A., Multari, S., Palaniappan, V., Ruggeri, L., Smeraldi, C., Tard, A., Castle, L., 2023. Safety evaluation of the food additive steviol glycosides, predominantly Rebaudioside M, produced by fermentation using *Yarrowia lipolytica* VRM. EFSA J. 21, e8387. https://doi.org/10.2903/j.efsa.2023.8387.
Elhalis, H., 2024. Expanding the horizons of *Saccharomyces cerevisiae*: nutrition, oenology, and bioethanol production. Sustainability 16, 11151. https://doi.org/10.3390/su162411151.
Emmerstorfer, A., Wimmer-Teubenbacher, M., Wriessnegger, T., Leitner, E., Müller, M., Kaluzna, I., Schürmann, M., Mink, D., Zellnig, G., Schwab, H., Pichler, H., 2015. Over-expression of *ICE2* stabilizes cytochrome P450 reductase in *Saccharomyces cerevisiae* and *Pichia pastoris*. Biotechnol. J. 10, 623–635. https://doi.org/10.1002/biot.201400780.
Gambacorta, F.V., Dietrich, J.J., Yan, Q., Pfleger, B.F., 2020. Rewiring yeast metabolism to synthesize products beyond ethanol. Curr. Opin. Chem. Biol. 59, 182–192. https://doi.org/10.1016/j.cbpa.2020.08.005.
Gao, J., Jiang, L., Lian, J., 2021. Development of synthetic biology tools to engineer *Pichia pastoris* as a chassis for the production of natural products. Synth. Syst. Biotechnol. 6, 110–119. https://doi.org/10.1016/j.synbio.2021.04.005.
Gao, Q., Wang, L., Zhang, M., Wei, Y., Lin, W., 2020. Recent advances on feasible strategies for monoterpenoid production in *Saccharomyces cerevisiae*. Front. Bioeng. Biotechnol. 8, 609800. https://doi.org/10.3389/fbioe.2020.609800.
Groenewald, M., Boekhout, T., Neuvéglise, C., Gaillardin, C., van Dijck, P.W.M., Wyss, M., 2014. *Yarrowia lipolytica*: safety assessment of an oleaginous yeast with a great industrial potential. Crit. Rev. Microbiol. 40, 187–206. https://doi.org/10.3109/1040841X.2013.770386.
Guo, H., Wang, H., Huo, Y.-X., 2020. Engineering critical enzymes and pathways for improved triterpenoid biosynthesis in yeast. ACS Synth. Biol. 9, 2214–2227. https://doi.org/10.1021/acssynbio.0c00124.
Guo, Q., Peng, Q.-Q., Li, Y.-W., Yan, F., Wang, Y.-T., Ye, C., Shi, T.-Q., 2024. Advances in the metabolic engineering of *Saccharomyces cerevisiae* and *Yarrowia lipolytica* for the production of β-carotene. Crit. Rev. Biotechnol. 44, 337–351. https://doi.org/10.1080/07388551.2023.2166809.
Guo, Q., Shi, T.-Q., Peng, Q.-Q., Sun, X.-M., Ji, X.-J., Huang, H., 2021. Harnessing *Yarrowia lipolytica* peroxisomes as a subcellular factory for α-humulene overproduction. J. Agric. Food Chem. 69, 13831–13837. https://doi.org/10.1021/acs.jafc.1c05897.
Heirendt, L., Arreckx, S., Pfau, T., Mendoza, S.N., Richelle, A., Heinken, A., Haraldsdóttir, H.S., Wachowiak, J., Keating, S.M., Vlasov, V., Magnusdóttir, S., Ng, C.Y., Preciat, G., Žagare, A., Chan, S.H.J., Aurich, M.K., Clancy, C.M., Modamio, J., Sauls, J.T., Noronha, A., Bordbar, A., Cousins, B., El Assal, D.C., Valcarcel, L.V., Apaolaza, I., Ghaderi, S., Ahookhosh, M., Ben Guebila, M., Kostromins, A., Sompairac, N., Le, H.M., Ma, D., Sun, Y., Wang, L., Yurkovich, J.T., Oliveira, M.A.P., Vuong, P.T., El Assal, L.P., Kuperstein, I., Zinovyev, A., Hinton, H.S., Bryant, W.A., Aragón Artacho, F.J., Planes, F.J., Stalidzans, E., Maass, A., Vempala, S., Hucka, M., Saunders, M.A., Maranas, C.D., Lewis, N.E., Sauter, T., Palsson, B.Ø., Thiele, I., Fleming, R.M.T., 2019. Creation and analysis of biochemical constraint-based models using the COBRA Toolbox v.3.0. Nat. Protoc. 14, 639–702. https://doi.org/10.1038/s41596-018-0098-2.
Huelin, F.E., Murray, K.E., 1966. Alpha-farnesene in the natural coating of apples. Nature 210, 1260–1261. https://doi.org/10.1038/2101260a0.
Ignea, C., Pontini, M., Maffei, M.E., Makris, A.M., Kampranis, S.C., 2014. Engineering monoterpene production in yeast using a synthetic dominant negative geranyl diphosphate synthase. ACS Synth. Biol. 3, 298–306. https://doi.org/10.1021/sb400115e.
Ignea, C., Raadam, M.H., Koutsaviti, A., Zhao, Y., Duan, Y.-T., Harizani, M., Miettinen, K., Georgantea, P., Rosenfeldt, M., Viejo-Ledesma, S.E., Petersen, M.A., Bredie, W.L.P., Staerk, D., Roussis, V., Ioannou, E., Kampranis, S.C., 2022. Expanding the terpene biosynthetic code with non-canonical 16 carbon atom building blocks. Nat. Commun. 13, 5188. https://doi.org/10.1038/s41467-022-32921-w.
Ignea, C., Raadam, M.H., Motawia, M.S., Makris, A.M., Vickers, C.E., Kampranis, S.C., 2019. Orthogonal monoterpenoid biosynthesis in yeast constructed on an isomeric substrate. Nat. Commun. 10, 3799. https://doi.org/10.1038/s41467-019-11290-x.
Ignea, C., Trikka, F.A., Nikolaidis, A.K., Georgantea, P., Ioannou, E., Loupassaki, S., Kefalas, P., Kanellis, A.K., Roussis, V., Makris, A.M., Kampranis, S.C., 2015. Efficient diterpene production in yeast by engineering Erg20p into a geranylgeranyl diphosphate synthase. Metab. Eng. 27, 65–75. https://doi.org/10.1016/j.ymben.2014.10.008.
Jaramillo-Madrid, A.C., Lacchini, E., Goossens, A., 2022. Within and beyond organelle engineering: strategies for increased terpene production in yeasts and plants. Curr. Opin. Green Sustain. Chem. 33, 100572. https://doi.org/10.1016/j.cogsc.2021.100572.
Jin, K., Xia, H., Liu, Y., Li, J., Du, G., Lv, X., Liu, L., 2022. Compartmentalization and transporter engineering strategies for terpenoid synthesis. Microb. Cell Fact. 21, 92. https://doi.org/10.1186/s12934-022-01819-z.
Jordá, T., Puig, S., 2020. Regulation of ergosterol biosynthesis in *Saccharomyces cerevisiae*. Genes (Basel) 11, 795. https://doi.org/10.3390/genes11070795.
Kanamoto, H., Nakamura, K., Misawa, N., 2021. Carotenoid production in oleaginous yeasts. Adv. Exp. Med. Biol. 1261, 153–163. https://doi.org/10.1007/978-981-15-7360-6_12.
Karaca, H., Kaya, M., Kapkac, H.A., Levent, S., Ozkay, Y., Ozan, S.D., Nielsen, J., Krivoruchko, A., 2024. Metabolic engineering of *saccharomyces cerevisiae* for enhanced taxadiene production. Microb. Cell Fact. 23, 241. https://doi.org/10.1186/s12934-024-02512-z.
Kastberg, L.L.B., Ard, R., Jensen, M.K., Workman, C.T., 2022. Burden imposed by heterologous protein production in two major industrial yeast cell factories: identifying sources and mitigation strategies. Front. Fungal. Biol. 3, 827704. https://doi.org/10.3389/ffunb.2022.827704.
Kim, J.-E., Jang, I.-S., Son, S.-H., Ko, Y.-J., Cho, B.-K., Kim, S.C., Lee, J.Y., 2019a. Tailoring the *Saccharomyces cerevisiae* endoplasmic reticulum for functional assembly of terpene synthesis pathway. Metab. Eng. 56, 50–59. https://doi.org/10.1016/j.ymben.2019.08.013.
Kim, S., Lee, J., Sung, B.H., 2019b. Isolation and characterization of the stress-tolerant *Candida tropicalis* YHJ1 and evaluation of its xylose reductase for xylitol production from acid pre-treatment wastewater. Front. Bioeng. Biotechnol. 7, 138. https://doi.org/10.3389/fbioe.2019.00138.

Kim, S.R., Ha, S.-J., Wei, N., Oh, E.J., Jin, Y.-S., 2012. Simultaneous co-fermentation of mixed sugars: a promising strategy for producing cellulosic ethanol. Trends Biotechnol. 30, 274–282. https://doi.org/10.1016/j.tibtech.2012.01.005.
Kong, J., Miao, L., Lu, Z., Wang, S., Zhao, B., Zhang, C., Xiao, D., Teo, D., Leong, S.S.J., Wong, A., Yu, A., 2022. Enhanced production of amyrin in *yarrowia lipolytica* using a combinatorial protein and metabolic engineering approach. Microb. Cell Fact. 21, 186. https://doi.org/10.1186/s12934-022-01915-0.
Kwak, S., Crook, N., Yoneda, A., Ahn, N., Ning, J., Cheng, J., Dantas, G., 2022. Functional mining of novel terpene synthases from metagenomes. Biotechnol. Biofuels. Bioprod. 15, 104. https://doi.org/10.1186/s13068-022-02189-9.
Kwak, S., Jo, J.H., Yun, E.J., Jin, Y.-S., Seo, J.-H., 2019. Production of biofuels and chemicals from xylose using native and engineered yeast strains. Biotechnol. Adv. 37, 271–283. https://doi.org/10.1016/j.biotechadv.2018.12.003.
Kwak, S., Kim, S.R., Xu, H., Zhang, G.-C., Lane, S., Kim, H., Jin, Y.-S., 2017. Enhanced isoprenoid production from xylose by engineered *Saccharomyces cerevisiae*. Biotechnol. Bioeng. 114, 2581–2591. https://doi.org/10.1002/bit.26369.
Kwak, S., Yun, E.J., Lane, S., Oh, E.J., Kim, K.H., Jin, Y.-S., 2020. Redirection of the glycolytic flux enhances isoprenoid production in *Saccharomyces cerevisiae*. Biotechnol. J. 15, e1900173. https://doi.org/10.1002/biot.201900173.
Lee, J.W., Yook, S., Koh, H., Rao, C.V., Jin, Y.-S., 2021. Engineering xylose metabolism in yeasts to produce biofuels and chemicals. Curr. Opin. Biotechnol. 67, 15–25. https://doi.org/10.1016/j.copbio.2020.10.012.
Li, D., Shen, J., Ding, Q., Wu, J., Chen, X., 2024a. Recent progress of atmospheric and room-temperature plasma as a new and promising mutagenesis technology. Cell Biochem. Funct. 42, e3991. https://doi.org/10.1002/cbf.3991.
Li, G., Liang, H., Gao, R., Qin, L., Xu, P., Huang, M., Zong, M.-H., Cao, Y., Lou, W.-Y., 2024b. Yeast metabolism adaptation for efficient terpenoids synthesis via isopentenol utilization. Nat. Commun. 15, 9844. https://doi.org/10.1038/s41467-024-54298-8.
Li, Jian, Zhu, K., Miao, L., Rong, L., Zhao, Y., Li, S., Ma, L., Li, J., Zhang, C., Xiao, D., Foo, J.L., Yu, A., 2021. Simultaneous improvement of limonene production and tolerance in *Yarrowia lipolytica* through tolerance engineering and evolutionary engineering. ACS Synth. Biol. 10, 884–896. https://doi.org/10.1021/acssynbio.1c00052.
Li, T., Liu, G.-S., Zhou, W., Jiang, M., Ren, Y.-H., Tao, X.-Y., Liu, M., Zhao, M., Wang, F.-Q., Gao, B., Wei, D.-Z., 2020a. Metabolic engineering of *Saccharomyces cerevisiae* to overproduce squalene. J. Agric. Food Chem. 68, 2132–2138. https://doi.org/10.1021/acs.jafc.9b07419.
Li, Y., Kong, D., Fu, Y., Sussman, M.R., Wu, H., 2020b. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol. Biochem. 148, 80–89. https://doi.org/10.1016/j.plaphy.2020.01.006.
Liang, J., Wang, D., Li, X., Huang, W., Xie, C., Fu, M., Zhang, H., Meng, Q., 2023. In silico genome-wide mining and analysis of terpene synthase gene family in *Hevea Brasiliensis*. Biochem. Genet. 61, 1185–1209. https://doi.org/10.1007/s10528-022-10311-7.
Liu, C.-L., Xue, K., Yang, Y., Liu, X., Li, Y., Lee, T.S., Bai, Z., Tan, T., 2022. Metabolic engineering strategies for sesquiterpene production in microorganism. Crit. Rev. Biotechnol. 42, 73–92. https://doi.org/10.1080/07388551.2021.1924112.
Liu, G.-S., Li, T., Zhou, W., Jiang, M., Tao, X.-Y., Liu, M., Zhao, M., Ren, Y.-H., Gao, B., Wang, F.-Q., Wei, D.-Z., 2020. The yeast peroxisome: a dynamic storage depot and subcellular factory for squalene overproduction. Metab. Eng. 57, 151–161. https://doi.org/10.1016/j.ymben.2019.11.001.
Liu, H., Chen, S.-L., Xu, J.-Z., Zhang, W.-G., 2021. Dual regulation of cytoplasm and peroxisomes for improved α-farnesene production in recombinant *Pichia pastoris*. ACS Synth. Biol. 10, 1563–1573. https://doi.org/10.1021/acssynbio.1c00186.
Liu, Z., Huang, M., Chen, H., Lu, X., Tian, Y., Hu, P., Zhao, Q., Li, P., Li, C., Ji, X., Liu, H., 2024a. Metabolic engineering of *Yarrowia lipolytica* for high-level production of squalene. Bioresour. Technol. 394, 130233. https://doi.org/10.1016/j.biortech.2023.130233.
Liu, Z., Huang, M., Chen, H., Lu, X., Tian, Y., Hu, P., Zhao, Q., Li, P., Li, C., Ji, X., Liu, H., 2024b. Metabolic engineering of *Yarrowia lipolytica* for high-level production of squalene. Bioresour. Technol. 394, 130233. https://doi.org/10.1016/j.biortech.2023.130233.
Long, Y., Han, X., Meng, X., Xu, P., Tao, F., 2024. A robust yeast chassis: comprehensive characterization of a fast-growing *saccharomyces cerevisiae*. mBio 15, e0319623. https://doi.org/10.1128/mbio.03196-23.
Lopes, A.M., Magalhães, P.O., Mazzola, P.G., Rangel-Yagui, C.O., de Carvalho, J.C.M., Penna, T.C.V., Pessoa, A., 2010. LPS removal from an *E. coli* fermentation broth using aqueous two-phase micellar system. Biotechnol. Prog. 26, 1644–1653. https://doi.org/10.1002/btpr.463.
Lu, S., Zhou, C., Guo, X., Du, Z., Cheng, Y., Wang, Z., He, X., 2022. Enhancing fluxes through the mevalonate pathway in *Saccharomyces cerevisiae* by engineering the HMGR and β-alanine metabolism. Microb. Biotechnol. 15, 2292–2306. https://doi.org/10.1111/1751-7915.14072.
Lynd, L.R., Liang, X., Biddy, M.J., Allee, A., Cai, H., Foust, T., Himmel, M.E., Laser, M.S., Wang, M., Wyman, C.E., 2017. Cellulosic ethanol: status and innovation. Curr. Opin. Biotechnol. 45, 202–211. https://doi.org/10.1016/j.copbio.2017.03.008.
Ma, Y., Shang, Y., Stephanopoulos, G., 2024. Engineering peroxisomal biosynthetic pathways for maximization of triterpene production in *Yarrowia lipolytica*. Proc. Natl. Acad. Sci. U S A 121, e2314798121. https://doi.org/10.1073/pnas.2314798121.
Mai, J., Li, W., Ledesma-Amaro, R., Ji, X.-J., 2021. Engineering plant sesquiterpene synthesis into yeasts: a review. J. Agric. Food Chem. 69, 9498–9510. https://doi.org/10.1021/acs.jafc.1c03864.
Malcı, K., Santibáñez, R., Jonguitud-Borrego, N., Santoyo-Garcia, J.H., Kerkhoven, E.J., Rios-Solis, L., 2023. Improved production of Taxol® precursors in *S. cerevisiae* using combinatorial in silico design and metabolic engineering. Microb. Cell Fact. 22, 243. https://doi.org/10.1186/s12934-023-02251-7.
Malina, C., Yu, R., Björkeroth, J., Kerkhoven, E.J., Nielsen, J., 2021. Adaptations in metabolism and protein translation give rise to the Crabtree effect in yeast. Proc. Natl. Acad. Sci. U S A 118, e2112836118. https://doi.org/10.1073/pnas.2112836118.
Mamat, U., Wilke, K., Bramhill, D., Schromm, A.B., Lindner, B., Kohl, T.A., Corchero, J. L., Villaverde, A., Schaffer, L., Head, S.R., Souvignier, C., Meredith, T.C., Woodard, R.W., 2015. Detoxifying *Escherichia coli* for endotoxin-free production of recombinant proteins. Microb. Cell Fact. 14, 57. https://doi.org/10.1186/s12934-015-0241-5.
Mantzouridou, F., Tsimidou, M.Z., 2010. Observations on squalene accumulation in *Saccharomyces cerevisiae* due to the manipulation of *HMG2* and *ERG6*. FEMS Yeast Res. 10, 699–707. https://doi.org/10.1111/j.1567-1364.2010.00645.x.
Martin, V.J.J., Pitera, D.J., Withers, S.T., Newman, J.D., Keasling, J.D., 2003. Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. Nat. Biotechnol. 21, 796–802. https://doi.org/10.1038/nbt833.
Mason, C.A., 1991. Physiological aspects of growth and recombinant DNA stability in *Saccharomyces cerevisiae*. Antonie Van Leeuwenhoek 59, 269–283. https://doi.org/10.1007/BF00583680.
Meadows, A.L., Hawkins, K.M., Tsegaye, Y., Antipov, E., Kim, Y., Raetz, L., Dahl, R.H., Tai, A., Mahatdejkul-Meadows, T., Xu, L., Zhao, L., Dasika, M.S., Murarka, A., Lenihan, J., Eng, D., Leng, J.S., Liu, C.-L., Wenger, J.W., Jiang, H., Chao, L., Westfall, P., Lai, J., Ganesan, S., Jackson, P., Mans, R., Platt, D., Reeves, C.D., Saija, P.R., Wichmann, G., Holmes, V.F., Benjamin, K., Hill, P.W., Gardner, T.S., Tsong, A.E., 2016. Rewriting yeast central carbon metabolism for industrial isoprenoid production. Nature 537, 694–697. https://doi.org/10.1038/nature19769.
Mendes de Lacerda Leite, G., de Oliveira Barbosa, M., Pereira Lopes, M.J., de Araújo Delmondes, G., Bezerra, D.S., Araújo, I.M., Carvalho de Alencar, C.D., Melo Coutinho, H.D., Peixoto, L.R., Barbosa-Filho, J.M., Bezerra Felipe, C.F., Barbosa, R., Alencar de Menezes, I.R., Kerntof, M.R., 2021. Pharmacological and toxicological activities of α-humulene and its isomers: a systematic review. Trends Food Sci. Technol. 115, 255–274. https://doi.org/10.1016/j.tifs.2021.06.049.
Meng, X., Liu, H., Xu, W., Zhang, W., Wang, Z., Liu, W., 2020. Metabolic engineering *Saccharomyces cerevisiae* for de novo production of the sesquiterpenoid (+)-nootkatone. Microb. Cell Fact. 19, 21. https://doi.org/10.1186/s12934-020-1295-6.
Mewalal, R., Rai, D.K., Kainer, D., Chen, F., Külheim, C., Peter, G.F., Tuskan, G.A., 2017. Plant-derived terpenes: a feedstock for specialty biofuels. Trends Biotechnol. 35, 227–240. https://doi.org/10.1016/j.tibtech.2016.08.003.
Micera, M., Botto, A., Geddo, F., Antoniotti, S., Bertea, C.M., Levi, R., Gallo, M.P., Querio, G., 2020. Squalene: more than a step toward sterols. Antioxidants 9, 688. https://doi.org/10.3390/antiox9080688.
Montanti, J., Nghiem, N.P., Johnston, D.B., 2011. Production of astaxanthin from cellulosic biomass sugars by mutants of the yeast *Phaffia rhodozyma*. Appl. Biochem. Biotechnol. 164, 655–665. https://doi.org/10.1007/s12010-011-9165-7.
Muhammad, A., Feng, X., Rasool, A., Sun, W., Li, C., 2020. Production of plant natural products through engineered *Yarrowia lipolytica*. Biotechnol. Adv. 43, 107555. https://doi.org/10.1016/j.biotechadv.2020.107555.
Mukherjee, M., Blair, R.H., Wang, Z.Q., 2022. Machine-learning guided elucidation of contribution of individual steps in the mevalonate pathway and construction of a yeast platform strain for terpenoid production. Metab. Eng. 74, 139–149. https://doi.org/10.1016/j.ymben.2022.10.004.
Mussagy, C.U., 2024. Advances in microbial astaxanthin production. Adv. Appl. Microbiol. 128, 105–120. https://doi.org/10.1016/bs.aambs.2024.05.001.
Naz, T., Ullah, S., Nazir, Y., Li, S., Iqbal, B., Liu, Q., Mohamed, H., Song, Y., 2023. Industrially important fungal carotenoids: advancements in biotechnological production and extraction. J. Fungi. 9, 578. https://doi.org/10.3390/jof9050578.
Nielsen, J., 2014. Synthetic biology for engineering acetyl coenzyme A metabolism in yeast. MBio 5, e02153. https://doi.org/10.1128/mBio.02153-14.
Nowrouzi, B., Li, R.A., Walls, L.E.D.', Espaux, L., Malcı, K., Liang, L., Jonguitud-Borrego, N., Lerma-Escalera, A.I., Morones-Ramirez, J.R., Keasling, J.D., Rios-Solis, L., 2020. Enhanced production of taxadiene in *Saccharomyces cerevisiae*. Microb. Cell Fact. 19, 200. https://doi.org/10.1186/s12934-020-01458-2.
Ohto, C., Muramatsu, M., Obata, S., Sakuradani, E., Shimizu, S., 2010. Production of geranylgeraniol on overexpression of a prenyl diphosphate synthase fusion gene in *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 87, 1327–1334. https://doi.org/10.1007/s00253-010-2571-x.
Paddon, C.J., Keasling, J.D., 2014. Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development. Nat. Rev. Microbiol. 12, 355–367. https://doi.org/10.1038/nrmicro3240.
Padella, M., O'Connell, A., Prussi, M., 2019. What is still limiting the deployment of cellulosic ethanol ? Analysis of the current status of the sector. Appl. Sci. 9, 4523. https://doi.org/10.3390/app9214523.
Pandit, A.V., Srinivasan, S., Mahadevan, R., 2017. Redesigning metabolism based on orthogonality principles. Nat. Commun. 8, 15188. https://doi.org/10.1038/ncomms15188.
Papagiannidis, D., Bircham, P.W., Lüchtenborg, C., Pajonk, O., Ruffini, G., Brügger, B., Schuck, S., 2021. Ice2 promotes ER membrane biogenesis in yeast by inhibiting the conserved lipin phosphatase complex. EMBO J. 40, e107958. https://doi.org/10.15252/embj.2021107958.
Parapouli, M., Vasileiadis, A., Afendra, A.-S., Hatziloukas, E., 2020. *Saccharomyces cerevisiae* and its industrial applications. AIMS Microbiol. 6, 1–31. https://doi.org/10.3934/microbiol.2020001.
Park, Y.-K., Sellés Vidal, L., Bell, D., Zabret, J., Soldat, M., Kzzavšček, M., Ledesma-Amaro, R., 2024. Efficient synthesis of limonene production in *Yarrowia lipolytica* by

combinatorial engineering strategies. Biotechnol. Biofuels. Bioprod. 17, 94. https://doi.org/10.1186/s13068-024-02535-z.
Peralta-Yahya, P.P., Ouellet, M., Chan, R., Mukhopadhyay, A., Keasling, J.D., Lee, T.S., 2011. Identification and microbial production of a terpene-based advanced biofuel. Nat. Commun. 2, 483. https://doi.org/10.1038/ncomms1494.
Qaderi, M.M., Martel, A.B., Strugnell, C.A., 2023. Environmental factors regulate plant secondary metabolites. Plants 12, 447. https://doi.org/10.3390/plants12030447.
Qu, Z., Zhang, L., Zhu, S., Yuan, W., Hang, J., Yin, D., Tang, X., Zheng, J., Wang, Z., Sun, J., 2020. Overexpression of the transcription factor *HAC1* improves nerolidol production in engineered yeast. Enzyme Microb. Technol. 134, 109485. https://doi.org/10.1016/j.enzmictec.2019.109485.
Raj, V., Venkataraman, B., Ojha, S.K., Almarzooqi, S., Subramanian, V.S., Al-Ramadi, B. K., Adrian, T.E., Subramanya, S.B., 2023. Cis- nerolidol inhibits MAP kinase and NF-κB signaling pathways and prevents epithelial tight junction dysfunction in colon inflammation: in vivo and in vitro studies. Molecules 28, 2982. https://doi.org/10.3390/molecules28072982.
Ravindran, R., Bacellar, I.O.L., Castellanos-Girouard, X., Wahba, H.M., Zhang, Z., Omichinski, J.G., Kisley, L., Michnick, S.W., 2023. Peroxisome biogenesis initiated by protein phase separation. Nature 617, 608–615. https://doi.org/10.1038/s41586-023-06044-1.
Ren, Y., Liu, S., Jin, G., Yang, X., Zhou, Y.J., 2020. Microbial production of limonene and its derivatives: achievements and perspectives. Biotechnol. Adv. 44, 107628. https://doi.org/10.1016/j.biotechadv.2020.107628.
Rudolf, J.D., Alsup, T.A., Xu, B., Li, Z., 2021. Bacterial terpenome. Nat. Prod. Rep. 38, 905–980. https://doi.org/10.1039/d0np00066c.
Rudolf, J.D., Chang, C.-Y., 2020. Terpene synthases in disguise: enzymology, structure, and opportunities of non-canonical terpene synthases. Nat. Prod. Rep. 37, 425–463. https://doi.org/10.1039/c9np00051h.
Rugbjerg, P., Sommer, M.O.A., 2019. Overcoming genetic heterogeneity in industrial fermentations. Nat. Biotechnol. 37, 869–876. https://doi.org/10.1038/s41587-019-0171-6.
Sandmann, G., 2022. Carotenoids and their biosynthesis in fungi. Molecules 27, 1431. https://doi.org/10.3390/molecules27041431.
Santos, F.A., Frota, J.T., Arruda, B.R., de Melo, T.S., da Silva, A.A., de, C.A., Brito, G.A., de, C., Chaves, M.H., Rao, V.S., 2012. Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice. Lipids Health Dis. 11, 98. https://doi.org/10.1186/1476-511X-11-98.
Schilmiller, A.L., Schauvinhold, I., Larson, M., Xu, R., Charbonneau, A.L., Schmidt, A., Wilkerson, C., Last, R.L., Pichersky, E., 2009. Monoterpenes in the glandular trichomes of tomato are synthesized from a neryl diphosphate precursor rather than geranyl diphosphate. Proc. Natl. Acad. Sci. U.S.A. 106, 10865–10870. https://doi.org/10.1073/pnas.0904113106.
Segal-Kischinevzky, C., Romero-Aguilar, L., Alcaraz, L.D., López-Ortiz, G., Martínez-Castillo, B., Torres-Ramírez, N., Sandoval, G., González, J., 2022. Yeasts inhabiting extreme environments and their biotechnological applications. Microorganisms 10, 794. https://doi.org/10.3390/microorganisms10040794.
Shi, B., Ma, T., Ye, Z., Li, X., Huang, Y., Zhou, Z., Ding, Y., Deng, Z., Liu, T., 2019. Systematic metabolic engineering of *Saccharomyces cerevisiae* for lycopene overproduction. J. Agric. Food Chem. 67, 11148–11157. https://doi.org/10.1021/acs.jafc.9b04519.
Shu, Y., Dong, T., Zhou, X., Wang, H., Liu, H., Yao, M., Wang, Y., Xiao, W., 2024. Systematic engineering to enhance β-myrcene production in yeast. J. Agric. Food Chem. 72, 19395–19402. https://doi.org/10.1021/acs.jafc.4c05046.
Simão da Silva, K.A.B., Paszcuk, A.F., Passos, G.F., Silva, E.S., Bento, A.F., Meotti, F.C., Calixto, J.B., 2011. Activation of cannabinoid receptors by the pentacyclic triterpene α,β-amyrin inhibits inflammatory and neuropathic persistent pain in mice. Pain 152, 1872–1887. https://doi.org/10.1016/j.pain.2011.04.005.
Su, S., Qin, S., Xia, H., Li, P., Li, H., Li, C., Guo, S., Zeng, C., 2024. The impact of oil type on the performance of β-amyrin-based oleogels: formation, physicochemical properties, and potential correlation analysis. Foods. 13, 876. https://doi.org/10.3390/foods13060876.
Surendran, S., Qassadi, F., Surendran, G., Lilley, D., Heinrich, M., 2021. Myrcene-what are the potential health benefits of this flavouring and aroma agent ? Front. Nutr. 8, 699666. https://doi.org/10.3389/fnut.2021.699666.
Taguchi, S., Ooi, T., Mizuno, K., Matsusaki, H., 2015. Advances and needs for endotoxin-free production strains. Appl. Microbiol. Biotechnol. 99, 9349–9360. https://doi.org/10.1007/s00253-015-6947-9.
Tang, W.-Y., Wang, D.-P., Tian, Y., Fan, X., Wang, C., Lu, X.-Y., Li, P.-W., Ji, X.-J., Liu, H.-H., 2021. Metabolic engineering of *Yarrowia lipolytica* for improving squalene production. Bioresour. Technol. 323, 124652. https://doi.org/10.1016/j.biortech.2020.124652.
Tao, H., Lauterbach, L., Bian, G., Chen, R., Hou, A., Mori, T., Cheng, S., Hu, B., Lu, L., Mu, X., Li, M., Adachi, N., Kawasaki, M., Moriya, T., Senda, T., Wang, X., Deng, Z., Abe, I., Dickschat, J.S., Liu, T., 2022. Discovery of non-squalene triterpenes. Nature 606, 414–419. https://doi.org/10.1038/s41586-022-04773-3.
Taton, M., Benveniste, P., Rahier, A., Johnson, W.S., Liu, H.T., Sudhakar, A.R., 1992. Inhibition of 2,3-oxidosqualene cyclases. Biochemistry 31, 7892–7898. https://doi.org/10.1021/bi00149a021.
Thangavelu, K., Sundararaju, P., Srinivasan, N., Muniraj, I., Uthandi, S., 2020. Simultaneous lipid production for biodiesel feedstock and decontamination of sago processing wastewater using *Candida tropicalis* ASY2. Biotechnol. Biofuels. 13, 35. https://doi.org/10.1186/s13068-020-01676-1.
Tomàs-Gamisans, M., Andrade, C.C.P., Maresca, F., Monforte, S., Ferrer, P., Albiol, J., 2020. Redox engineering by ectopic overexpression of NADH kinase in recombinant *Pichia pastoris* (*Komagataella phaffii*): impact on cell physiology and recombinant production of secreted proteins. Appl. Environ. Microbiol. 86, e02038. https://doi.org/10.1128/AEM.02038-19. -19.
Van Gelder, K., Lindner, S.N., Hanson, A.D., Zhou, J., 2024. Strangers in a foreign land: "yeastizing" plant enzymes. Microb. Biotechnol. 17, e14525. https://doi.org/10.1111/1751-7915.14525.
Vandermies, M., Fickers, P., 2019. Bioreactor- scale strategies for the production of recombinant protein in the yeast *Yarrowia lipolytica*. Microorganisms. 7, 40. https://doi.org/10.3390/microorganisms7020040.
Vargas-Sinisterra, A.F., Ramírez-Castrillón, M., 2021. Yeast carotenoids: production and activity as antimicrobial biomolecule. Arch. Microbiol. 203, 873–888. https://doi.org/10.1007/s00203-020-02111-7.
Vieira, E.F., Delerue-Matos, C., 2020. Exploitation of *Saccharomyces cerevisiae* enzymes in food processing and preparation of nutraceuticals and pharmaceuticals. In: Arora, N. K., Mishra, J., Mishra, V. (Eds.), Microbial Enzymes: Roles and Applications in Industries. Springer, Singapore, pp. 41–62. https://doi.org/10.1007/978-981-15-1710-5_2.
Vieira Gomes, A.M., Souza Carmo, T., Silva Carvalho, L., Mendonça Bahia, F., Parachin, N.S., 2018. Comparison of yeasts as hosts for recombinant protein production. Microorganisms 6, 38. https://doi.org/10.3390/microorganisms6020038.
Walls, L.E., Rios-Solis, L., 2020. Sustainable production of microbial isoprenoid derived advanced biojet fuels using different generation feedstocks: a review. Front. Bioeng. Biotechnol. 8, 599560. https://doi.org/10.3389/fbioe.2020.599560.
Wang, C., Liwei, M., Park, J.-B., Jeong, S.-H., Wei, G., Wang, Y., Kim, S.-W., 2018. Microbial platform for terpenoid production: *Escherichia coli* and yeast. Front. Microbiol. 9, 2460. https://doi.org/10.3389/fmicb.2018.02460.
Wang, J., Jiang, W., Liang, C., Zhu, L., Li, Y., Mo, Q., Xu, S., Chu, A., Zhang, L., Ding, Z., Shi, G., 2021. Overproduction of α-farnesene in *Saccharomyces cerevisiae* by farnesene synthase screening and metabolic engineering. J. Agric. Food Chem. 69, 3103–3113. https://doi.org/10.1021/acs.jafc.1c00008.
Wang, J., Kunze, M., Villoria-González, A., Weinhofer, I., Berger, J., 2024. Peroxisomal localization of a truncated HMG-CoA reductase under low cholesterol conditions. Biomolecules 14, 244. https://doi.org/10.3390/biom14020244.
Wang, J., Li, Y., Jiang, W., Hu, J., Gu, Z., Xu, S., Zhang, L., Ding, Z., Chen, W., Shi, G., 2023. Engineering *saccharomyces cerevisiae* YPH499 for overproduction of geranylgeraniol. J. Agric. Food Chem. 71, 9804–9814. https://doi.org/10.1021/acs.jafc.3c01820.
Wegner, S.A., Chen, J.-M., Ip, S.S., Zhang, Y., Dugar, D., Avalos, J.L., 2021. Engineering acetyl-CoA supply and *ERG9* repression to enhance mevalonate production in *Saccharomyces cerevisiae*. J. Ind. Microbiol. Biotechnol. 48, kuab050. https://doi.org/10.1093/jimb/kuab050.
Wei, L.-J., Cao, X., Liu, J.-J., Kwak, S., Jin, Y.-S., Wang, W., Hua, Q., 2021. Increased accumulation of squalene in engineered *Yarrowia lipolytica* through deletion of *PEX10* and *URE2*. Appl. Environ. Microbiol. 87, e0048121. https://doi.org/10.1128/AEM.00481-21.
Xia, L., Lv, Y., Liu, S., Yu, S., Zeng, W., Zhou, J., 2022. Enhancing squalene production in *Saccharomyces cerevisiae* by metabolic engineering and random mutagenesis. Front. Chem. Eng. 3, 790261. https://doi.org/10.3389/fceng.2021.790261.
Xu, J., 2021. Is natural population of *Candida tropicalis* sexual, parasexual, and/or asexual? Front. Cell Infect. Microbiol. 11, 751676. https://doi.org/10.3389/fcimb.2021.751676.
Yamada, Y., Kuzuyama, T., Komatsu, M., Shin-Ya, K., Omura, S., Cane, D.E., Ikeda, H., 2015. Terpene synthases are widely distributed in bacteria. Proc. Natl. Acad. Sci. U.S.A. 112, 857–862. https://doi.org/10.1073/pnas.1422108112.
Yanagibashi, S., Bamba, T., Kirisako, T., Kondo, A., Hasunuma, T., 2024. Beneficial effect of optimizing the expression balance of the mevalonate pathway introduced into the mitochondria on terpenoid production in *Saccharomyces cerevisiae*. J. Biosci. Bioeng. 137, 16–23. https://doi.org/10.1016/j.jbiosc.2023.11.004.
Ye, C., Li, M., Gao, J., Zuo, Y., Xiao, F., Jiang, X., Cheng, J., Huang, L., Xu, Z., Lian, J., 2024. Metabolic engineering of *Pichia pastoris* for overproduction of cis-trans nepetalactol. Metab. Eng. 84, 83–94. https://doi.org/10.1016/j.ymben.2024.06.007.
Ye, Z., Huang, Y., Shi, B., Xiang, Z., Tian, Z., Huang, M., Wu, L., Deng, Z., Shen, K., Liu, T., 2022. Coupling cell growth and biochemical pathway induction in *Saccharomyces cerevisiae* for production of (+)-valencene and its chemical conversion to (+)-nootkatone. Metab. Eng. 72, 107–115. https://doi.org/10.1016/j.ymben.2022.03.005.
Yu, T., Zhou, Y.J., Huang, M., Liu, Q., Pereira, R., David, F., Nielsen, J., 2018. Reprogramming yeast metabolism from alcoholic fermentation to lipogenesis. Cell 174, 1549–1558. https://doi.org/10.1016/j.cell.2018.07.013 e14.
Zeng, W., Jiang, Y., Shan, X., Zhou, J., 2023. Engineering *Saccharomyces cerevisiae* for synthesis of β-myrcene and (E)-β-ocimene. 3 Biotech 13, 384. https://doi.org/10.1007/s13205-023-03818-2.
Zhang, C., Chen, C., Bian, X., Zhang, J., Zhang, Z., Ma, Y., Lu, W., 2024. Construction of an orthogonal transport system for *saccharomyces cerevisiae* peroxisome to efficiently produce sesquiterpenes. Metab. Eng. 85, 84–93. https://doi.org/10.1016/j.ymben.2024.07.010.
Zhang, C., Chen, W., Dong, T., Wang, Y., Yao, M., Xiao, W., Li, B., 2023a. Elimination of enzymes catalysis compartmentalization enhancing taxadiene production in *Saccharomyces cerevisiae*. Front. Bioeng. Biotechnol. 11, 1141272. https://doi.org/10.3389/fbioe.2023.1141272.
Zhang, C., Hong, K., 2020. Production of terpenoids by synthetic biology approaches. Front. Bioeng. Biotechnol. 8, 347. https://doi.org/10.3389/fbioe.2020.00347.
Zhang, C., Li, M., Zhao, G.-R., Lu, W., 2020. Harnessing yeast peroxisomes and cytosol acetyl-coa for sesquiterpene α-humulene production. J. Agric. Food Chem. 68, 1382–1389. https://doi.org/10.1021/acs.jafc.9b07290.

Zhang, C., Liu, J., Zhao, F., Lu, C., Zhao, G.-R., Lu, W., 2018. Production of sesquiterpenoid zerumbone from metabolic engineered *Saccharomyces cerevisiae*. Metab. Eng. 49, 28–35. https://doi.org/10.1016/j.ymben.2018.07.010.

Zhang, G., Wang, H., Zhang, Z., Verstrepen, K.J., Wang, Q., Dai, Z., 2022. Metabolic engineering of *Yarrowia lipolytica* for terpenoids production: advances and perspectives. Crit. Rev. Biotechnol. 42, 618–633. https://doi.org/10.1080/07388551.2021.1947183.

Zhang, G.-C., Liu, J.-J., Kong, I.I., Kwak, S., Jin, Y.-S., 2015. Combining C6 and C5 sugar metabolism for enhancing microbial bioconversion. Curr. Opin. Chem. Biol. 29, 49–57. https://doi.org/10.1016/j.cbpa.2015.09.008.

Zhang, Q.-W., Kong, C.-L., Tao, Y.-S., 2023b. Fate of carotenoids in yeasts: synthesis and cleavage. Crit. Rev. Food Sci. Nutr. 63, 7638–7652. https://doi.org/10.1080/10408398.2022.2048352.

Zhang, T.-L., Yu, H.-W., Ye, L.-D., 2023c. Metabolic engineering of *Yarrowia lipolytica* for terpenoid production: tools and strategies. ACS Synth. Biol. 12, 639–656. https://doi.org/10.1021/acssynbio.2c00569.

Zhang, X., Liu, X., Meng, Y., Zhang, L., Qiao, J., Zhao, G.-R., 2021. Combinatorial engineering of *Saccharomyces cerevisiae* for improving limonene production. Biochem. Eng. J. 176, 108155. https://doi.org/10.1016/j.bej.2021.108155.

Zhang, Y., Cao, X., Wang, J., Tang, F., 2022c. Enhancement of linalool production in *Saccharomyces cerevisiae* by utilizing isopentenol utilization pathway. Microb. Cell Fact. 21, 212. https://doi.org/10.1186/s12934-022-01934-x.

Zhang, Y., Su, M., Wang, Z., Nielsen, J., Liu, Z., 2022d. Rewiring regulation on respiro-fermentative metabolism relieved Crabtree effects in *Saccharomyces cerevisiae*. Synth. Syst. Biotechnol. 7, 1034–1043. https://doi.org/10.1016/j.synbio.2022.06.004.

Zhou, J., Xie, X., Tang, H., Peng, C., Peng, F., 2022. The bioactivities of sclareol: a mini review. Front. Pharmacol. 13, 1014105. https://doi.org/10.3389/fphar.2022.1014105.

Zhou, P., Du, Y., Fang, X., Xu, N., Yue, C., Ye, L., 2021. Combinatorial modulation of linalool synthase and farnesyl diphosphate synthase for linalool overproduction in *Saccharomyces cerevisiae*. J. Agric. Food Chem. 69, 1003–1010. https://doi.org/10.1021/acs.jafc.0c06384.

Zhou, P., Du, Y., Xu, N., Yue, C., Ye, L., 2020. Improved linalool production in *Saccharomyces cerevisiae* by combining directed evolution of linalool synthase and overexpression of the complete mevalonate pathway. Biochem. Eng. J. 161, 107655. https://doi.org/10.1016/j.bej.2020.107655.

Zhou, Y.J., Gao, W., Rong, Q., Jin, G., Chu, H., Liu, W., Yang, W., Zhu, Z., Li, G., Zhu, G., Huang, L., Zhao, Z.K., 2012. Modular pathway engineering of diterpenoid synthases and the mevalonic acid pathway for miltiradiene production. J. Am. Chem. Soc. 134, 3234–3241. https://doi.org/10.1021/ja2114486.

Zhu, K., Kong, J., Zhao, B., Rong, L., Liu, S., Lu, Z., Zhang, C., Xiao, D., Pushpanathan, K., Foo, J.L., Wong, A., Yu, A., 2021. Metabolic engineering of microbes for monoterpenoid production. Biotechnol. Adv. 53, 107837. https://doi.org/10.1016/j.biotechadv.2021.107837.

Zielińska-Błajet, M., Feder-Kubis, J., 2020. Monoterpenes and their derivatives-recent development in biological and medical applications. Int. J. Mol. Sci. 21, 7078. https://doi.org/10.3390/ijms21197078.

Zuo, Y., Xiao, F., Gao, J., Ye, C., Jiang, L., Dong, C., Lian, J., 2022. Establishing *Komagataella phaffii* as a cell factory for efficient production of sesquiterpenoid α-santalene. J. Agric. Food Chem. 70, 8024–8031. https://doi.org/10.1021/acs.jafc.2c02353.