

Review: Technical Production Methods of Precision-Fermented Foods Identification and Characterisation of Hazards

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FSA Research and Evidence

This review examined production methods for precision fermentation of food ingredients, using the FSA's working definition centred on genetically modified micro-organisms producing functional components.

It maps host selection (yeasts, filamentous fungi, selected bacteria), genetic design (CRISPR, omics, adaptive evolution), upstream operations (media, alternative feedstocks, cell banking, seed trains, bioreactors) and downstream purification (harvesting, extraction, membranes, chromatography, stabilisation). It identifies risk clusters: chemical contaminants and residues; microbial contamination and host-cell remnants; GMM-specific issues such as toxins and horizontal gene transfer; and product-related concerns including allergenicity and unintended bioactivity.

The review concludes that proportionate, multi-layered risk assessment, controls and oversight are essential.

1. Introduction

Climate change and population growth are key drivers placing increasing pressure on global food system. Meeting future food demand requires balancing the need for increased production with efforts to reduce the environmental impact of agricultural practices. Increasing consumer expectations for safe, healthy and ethically produced food are contributing to the growing interest in alternative proteins and cellular agriculture. Precision fermentation (PF) has emerged as a promising technology in the alternative protein production using microorganism (yeast, bacteria, fungi, algae) selected and engineered to produce specific food ingredients (e.g., proteins, lipids, flavouring, enzymes) that are traditionally derived from animal or plant sources.

In 2024, the Good Food Institute identified that 165 companies focused on fermentation for alternative proteins ranging from animal-free dairy proteins to egg and collagen analogues as well as bioflavouring and

functional additives (Good Food Institute, 2024). While PF offers significant benefits, including improved efficiency, product variety and sustainability, its products still require thorough safety assessments, hazard mitigations strategies and regulatory oversight to ensure consumer safety and market acceptance.

The working definition of precision fermentation (PF) currently used by the Food Standards Agency (FSA) is “Precision fermentation is considered to be a technology that creates essential food components, like proteins and enzymes, through use of genetically modified microorganisms to produce specific functional components”. A fuller definition includes “where whole micro-organisms are reproduced in a bioreactor / closed system and refined for their fat or protein content”. To define the scope of this review, only the working definition of precision fermentation was considered.

This review examines key stages of precision fermentation, from microbial host selection and genetic design to upstream and downstream processing. It also identifies potential hazards associated with these processes. By reviewing current scientific literature and industry practices, it provides a comprehensive overview of the technological foundations and their implications for food safety and regulatory oversight.

2. Methodology

The review focused on microbial host selection, genetic design and optimization, upstream and downstream processing and potential food safety hazards associated with precision fermentation. The objective was to provide a comprehensive overview of technological production methods and regulatory considerations and identify associated food safety hazards. Literature was gathered from peer-reviewed journals, regulatory guidance documents and industry reports. Searches were performed using databases such as Google Scholar and PubMed, as well as regulatory sources like EFSA and FDA publications. Titles and abstracts were screened for relevance, followed by full text review. Data were organised into themes covering production methods, hazards characterizations (chemical, microbial and related to genetic modification of microorganisms) and regulatory consideration to identify common practice, emerging trends and safety implications.

It should be noted that this review summarises information from the literature and does not imply regulatory approval. Any technology described would require appropriate authorization in the country of sale. Differences in international regulatory frameworks were not considered here, as the aim was to capture a broad view of technological approaches and associated hazards. This includes uses of the technology that may fall outside the scope of Novel Food regulation in the country of sale, as detailed in some examples provided.

3. Technical Production Methods

3.1. Microbial Host Selection

Precision fermentation refers to the targeted use of genetically engineered microorganisms such as bacteria, yeasts and filamentous fungi to produce high-value biomolecules, including proteins, enzymes, lipids, pigments and other metabolites (Knychala et al., 2024). Selecting an appropriate microbial host is a critical first step in production of low-cost proteins. Host selection influences carbon yield, productivity, titre and overall process economics (Nielsen et al., 2024). Strain optimization strategies such as eliminating unwanted pathways or increasing gene copy numbers can significantly improve yields (Arnau et al., 2020; Dupuis et al., 2023). Commercially developed strains often incorporate enhancements for stability, performance and safety. Industrial-scale production typically utilises yeast and filamentous fungi (e.g., *Aspergillus niger*, *Aspergillus oryzae*, *Kluyveromyces lactis*, *Komagataella phaffii*, *Saccharomyces cerevisiae*, *T. reesei*, and *Yarrowia lipolytica*), and some bacterial species like *Bacillus spp.* (Chai et al., 2022; Pereira et al., 2025). These hosts, referred to as “cell factories”, differ in capabilities:

- Filamentous fungi excel at protein secretion, growth on inexpensive substrates and post translation modification, reducing downstream costs. However, their slower growth increases bioreactor residence time and tank size requirements (Ntana et al., 2020).
- Bacteria and yeasts such as *Escherichia coli* and *S. cerevisiae* grow rapidly and offer comprehensive genetic toolboxes, but many suffer from low yields, limited post-translation modifications, and challenging recovery (Nielsen et al., 2024; Ntana et al., 2020).
- Animal cell cultures remain impractical as molecular machines to produce food proteins due to slow growth and costly media.

The host selection process starts by clearly defining the target molecule (e.g., enzyme, protein, lipid) and its end use requirements, such as regulatory framework, purity and production scale. This initial step is critical because it informs all subsequent design decision, such as whether the product requires post-translation modification (PTMs), efficient secretion, or appropriate folding. For example, simple metabolites or enzymes that do not require PTMs are often produced in prokaryotic hosts like *Escherichia coli*, offering rapid growth and cost efficiency. In contrast, complex eukaryotic proteins such as caseins or collagen typically demand

hosts capable of performing PTMs and proper folding, such as *Komagataella phaffii* (formerly *Pichia pastoris*) or *Saccharomyces cerevisiae* (Karbalaie et al., 2020; Knychala et al., 2024).

Selecting an optimal microbial host for precision fermentation requires considering multiple criteria that influence regulatory compliance, efficiency and economic viability. Each factor plays a critical role in determining whether a host can deliver consistent performance from laboratory to commercial production while meeting quality and regulatory compliance. The key criteria and their implications for host selection are outlined below:

Safety and regulatory acceptance

Ensuring safety is fundamental in industrial biotechnological applications, particularly when products are intended for food, feed or cosmetic applications. Microorganism with Qualified Presumption of Safety (QPS) or Generally Recognised as Safe (GRAS) status, or those with a well-documented history of a safe use, are favoured as they generally present less risk and reduce the complexity of the regulatory process while also strengthening consumer trust and acceptance.

Genetic Traceability and Availability of Engineering Tools

Hosts equipped with robust genetic toolkits such as transformation systems, plasmid vectors, and clustered regularly interspaced short palindromic repeats (CRISPR/Cas) platforms, enable faster strain development and optimization (Y. Li & Smolke, 2016; Wen et al., 2024). For instance, *Escherichia Coli* and *Saccharomyces cerevisiae* offer extensive libraries and genome-editing tools, while *Pseudomonas putida* supports efficient CRISPR system for rapid genome editing (Wen et al., 2024).

Growth Rate and Cultivation Robustness

Industrial hosts must combine rapid growth with resilience to process related stresses (such as temperature, pH and mechanical shear) while sustaining high productivity under large-scale fermentation conditions. These traits are essential for minimizing downtime, reducing operational costs and ensuring consistency in product quality.

Product Compatibility and Post-Translational Modification

The nature of the target molecule plays a critical role in host selection. Prokaryotic systems lack complex PTMs, which limits their suitability for producing eukaryotic proteins that require glycosylation or disulfide bond

formation (Knychala et al., 2024). In contrast, yeasts and filamentous fungi are capable of performing these modifications, making them suitable hosts for the production of recombinant enzymes and animal protein analogues (Karbalaie et al., 2020).

Secretion Capability and Downstream Simplification

Hosts capable of secreting recombinant proteins directly into the culture medium significantly simplify downstream processing. Yeasts such as *Komagataella phaffii* and filamentous fungi like *Aspergillus niger* secrete proteins, reducing purification costs and increasing titre (Knychala et al., 2024). However, some filamentous fungi also release protease that can degrade the target product, which must be managed during process design.

Substrate Utilization and Feedstock Flexibility

Economic feasibility of precision fermentation depends on the ability to use inexpensive substrates such as agricultural residues or waste glycerol. Comparison of *E. coli*, *S. cerevisiae*, and *A. niger* showed significant differences in carbon-source utilization and inhibitor tolerance (Rumbold et al., 2009). Rumbold *et al.* found that fungi grew on all tested lignocellulosic feedstock hydrolysates, whereas bacteria failed to grow on willow wood hydrolysate and biodiesel-derived glycerol. The latter was also not utilised by *S. cerevisiae* and only poorly by *P. stipitis*. *T. reesei* grew well on crude but not on pure glycerol. The high sodium chloride levels in crude glycerol likely inhibited growth of *E. coli*, *C. glutamicum* and *S. cerevisiae*. Hosts like *Yarrowia lipolytica* can metabolise hydrophobic substrates, making them attractive for lipid-based product synthesis (Fickers et al., 2005).

Scalability and Industrial Robustness

Hosts must perform consistently from laboratory to industrial scale. Scale-up is one of the most challenging and complex stages in fermentation process. It requires careful consideration of factors that maintain process integrity during transition from laboratory to industrial scale. These include physical aspects (e.g., heat and mass transfer such as oxygen transfer rates and mixing time), biochemical factors (e.g., medium composition and rheology), and process-related elements (e.g., pre-culture and inoculum condition) (Mahdinia et al., 2019).

Fungi are key industrial microorganisms due to their adaptability and eukaryotic nature, making them ideal hosts for precision fermentation (Chai et al., 2022). Compared to bacteria, fungi can express heterologous eukaryotic proteins and enzymes and often accumulate valuable compounds such as organic acids, carotenoids and pigments (Lyu et al., 2019). Fungi can be classified as yeasts, unicellular fungi without hyphae

production, and filamentous fungi – moulds and macrofungi, which form mycelial networks and degrade recalcitrant biopolymers via extracellular enzymatic hydrolysis. This enzyme-secreting capacity, combined with pH and thermal stability, makes moulds responsible for over 50% of industrial enzyme production (Deckers et al., 2020).

Model yeasts include *Saccharomyces cerevisiae*, widely used for bread, beer and wine, with fully sequenced genome, available comprehensive metabolic models and GRAS status, and *Komagataella phaffii*, known for recombinant protein secretion (Chai et al., 2022). Filamentous fungi such as *Aspergillus spp.* are historically used in wine, vinegar and fermented food production. *Trichoderma reesei* are heavily utilised in food fermentation and biofuel industries as well as production of various food enzymes such as cellulases, carbohydrases, proteases, lipases, and lysozymes, while macrofungi are emerging platforms for CRISPR-based protein production (Waltz, 2016). Precision fermentation innovation relies on engineered strains, synthetic biology, and metabolic engineering to produce proteins, dairy alternatives, and speciality ingredients (Augustin et al., 2024; Ivey et al., 2021; Y. P. Zhang et al., 2017).

[Table 1](#) provides an overview of genetically modified microorganism employed in the production of diverse food ingredients. It highlights the type of organism, engineered traits, fermentation conditions and specific applications in food systems. These examples illustrate how advances in metabolic engineering and synthetic biology enable the synthesis of functional compounds such as amino acids, colorants, flavourings and bioactive molecules.

3.2. Genetic Design and Optimisation

The development of microbial hosts as efficient cell factories relies on a comprehensive set of genetic engineering and synthetic biology tools that enable precise control over metabolic pathways, protein expression and cellular physiology. These tools are essential for producing high-value compounds under industrial precision fermentation conditions while meeting safety and regulatory requirements. Advanced tools such as CRISPR-Cas systems, homologous recombination and transport mutagenesis enable precise genetic modifications.

CRISPR-Cas systems have transformed microbial genetic engineering by enabling precise, efficient, and cost-effective genome editing in bacteria, fungi and oomycetes (P. Yang et al., 2024). It enables precise manipulation of DNA sequences through targeted knockouts, insertions and replacements. Unlike earlier tools such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR requires only a guide RNA and Cas nuclease, making it simpler and highly versatile

Table 1. Genetically Modified Microorganisms Used to Produce Different Food Ingredients

Type of microorganism	Species	Products	Characteristics of the strain	Fermentation Conditions	Specific Applications in food	Reference
Bacteria	<i>Bacillus sp.</i>	Chitinase	Engineered for chitin and polysaccharide breakdown	LB media, 30 µg/L tetracycline, 37°C, 200 rpm, 12 h, 1.96 U/mL of chitinase activity	Used in food processing for texture and digestibility improvement	Yuan et al., 2023
		Lipopeptides (iturin A, fengycin, surfactin)	Optimised for antimicrobial lipopeptides	Landy medium, 37°C, 120 rpm, 120 h, levels of iturin A, fengycin and surfactin were: 67.75, 39.32 and 37.25 mg/L respectively	Food products biopreservation and natural food preservative	Chen et al., 2022
		Lycopene	Engineered for high yield	Soybean as substrate, 37°C, 5% inoculum, lycopene concentration (7.3 mg/kg of substrate)	Natural food colorant and antioxidant	D. Zou et al., 2022
	<i>Escherichia coli</i>	2-Keto-L-gulonic acid	Engineered for high yield; aerobic fermentation	48 h, 37°C, 2% inoculum, 220 rpm, 5.03 g/L of KGL acid obtained	Vitamin C and its derivatives biosynthesis	D. Li et al., 2023
		D-Pantothenic acid	Optimised for B-vitamin production	150 rpm, 37°C, 48 h, specific productivity of 0.79 g/L/h	Use in fortified foods and beverages	S. Zou et al., 2021
		Indigoidine	Overexpression of indigoidine biosynthetic genes	LB broth with addition of L-glutamine, 18°C, 250 rpm, 28 h, batch reactor, 8.81 g/L of indigoidine,	Natural colorant for food and drinks	Xu et al., 2015
		L-Valine	High-yield amino acid production	37°C, pH 7, 200 rpm, 15% inoculum, 84 g/L of L-Valine	Protein supplements and fortifiers	Hao et al., 2020
		Limonene	Optimization of MVA pathway genes	Glycerol as carbon source, 30°C, pO ₂ 30%, pH 7.2, fed-batch, 7.3 g/L of limonene	Flavouring in food, cosmetics and pharmaceuticals	Rolf et al., 2020
		β-Lactoglobulin	Engineered for high-purity whey protein production	37°C, 200 rpm, LB medium, 400 mg/L of β-Lactoglobulin	Functional food protein	Brune et al., 2023
		Violacein	Introduction of violacein cluster genes	Glycerol substrate, 30°C, 200 rpm, pH 6.8, batch reactor, 1.36 g/L of violacein	Natural colorant	D. Yang et al., 2021
		Vanillin	Overexpression of tyrosine biosynthetic genes with	Medium (10 g/L glucose, 5 g/L yeast extract, 10 g/L peptone, 5 g/L beef extract, and 2 g/L NaCl)	Natural flavouring for desserts,	Ni et al., 2015

Type of microorganism	Species	Products	Characteristics of the strain	Fermentation Conditions	Specific Applications in food	Reference
Filamentous fungi	<i>Aspergillus oryzae</i>	Kojic acid	vanillin conversion pathway Adapted to oxidative fermentation using H ₂ O ₂ for kojic acid production	fed with glucose, batch, 19.3 mg/L CM agar, 10–20 mM H ₂ O ₂ , 7 days 250 rpm, 33 °C, 32.5 g/L kojic acid,	beverages, bakery Natural preservative for food and cosmetics	X. Zhang et al., 2024
		Itaconic acid	High citric acid capacity; improved itaconate pathway	Citric acid substrate, 104 h, 35°C, 5% inoculum, 7.2 g/L of Itaconic acid	Bio-based food additives and acidulants	Xie et al., 2020
	<i>Aspergillus niger</i>	β-Glucan	Adapted for large-scale production with high purity	pH 5.5, 30°C, 72 h, 85.1% purity	Dietary fibre for gut health	Y. Li et al., 2023
Microalgae	<i>Chlamydomonas reinhardtii</i>	Lutein; β-Carotene	Production of carotenoids	YM broth, 75 μmol m ⁻² s ⁻¹ , 20°C, 22.8 (β-carotene); 8.9 (lutein) mg/ gDCW	Natural colorant and use in health supplements	Rathod et al., 2020
		Zeaxanthin	CRISPR-Cas9 was used to disrupt competing pathways and overexpression of β-carotene hydroxylase enabled efficient hydroxylation of β-carotene and increasing zeaxanthin concentration	TAP medium with 0.105 M acetate, 98 h under LED light at intensity of 80–100 μmol photons m ⁻² s ⁻¹ , 25°C, 21.68± 0.90 mg/L	Eye health supplements	Jang et al., 2025
	<i>Haematococcus sp.</i>	Astaxanthin	Production of astaxanthin, especially under stress conditions like high light intensity or nutrient limitation.	NIES-N medium, pH 7.5, 23°C, UV mutagenesis, 174 mg/L of astaxanthin	Colorant in aquaculture feed, functional food additive and cosmetics	Hong et al., 2018
Yeast	<i>Komagataella phaffii</i>	Xylitol	Naturally metabolises xylose; high pH and osmotic stress tolerance	Pure D-xylose substrate, 30°C, 50 mM KPi (pH 7), 80% (w/w) conversion within two hours	Low-calorie sweetener for diabetic-friendly foods and beverages.	Louie et al., 2021
		β-Alanine	Engineered for β-alanine production; robust under fed-batch	30°C, pH 6, fed batch reactor, 5.6 g/L β-alanine	Precursor for vitamin B5 synthesis, fortified food products	Miao et al., 2021

Type of microorganism	Species	Products	Characteristics of the strain	Fermentation Conditions	Specific Applications in food	Reference
	<i>Rhodospiridium toruloides</i>	Indigoidine	Introduction of indigoidine biosynthetic genes	Carbon and nitrogen feed (600g/L glucose and 63.6 g/L urea), 30°C, dissolved oxygen 30%, 400-900 rpm pH 7, 18.04 g/L of indigoidine	Natural colorant for food and drinks	Wehrs et al., 2019
	<i>Saccharomyces cerevisiae</i>	Caffeic acid	Strain modified to produce caffeic acid from tyrosine	Tyrosine substrate, 30°C, 250 rpm, 96 h, 289.4 mg/L of caffeic acid	Potential food antioxidant additive	Liu et al., 2019
		Indigoidine	Introduction of indigoidine biosynthetic genes	Glucose as carbon feed, dissolved oxygen 30% saturation, 400-600 rpm, 30°C, pH 6.6, feed-batch, 0.98 g/l of indigoidine	Natural colorant for food and drinks	Wehrs et al., 2018
		Lycopene	Modified for high lycopene synthesis; enhanced carotenoid pathway efficiency	3 L medium, 10% inoculum, 30°C, 3.28 g/L of lycopene	Natural colorant and antioxidant	Shi et al., 2019
		Resveratrol	Optimised for antioxidant production	Coumaric acid substrate, 391 mg/L of resveratrol	Preservative for functional foods	Sydor et al., 2010
		Vanillin	Engineered for vanillin biosynthesis; converts YPD medium into flavour precursors	1% inoculum, 50 mL YPD medium, 36 h, 30°C, 220 rpm, 8545 µg/L of vanillin,	Natural flavouring for desserts, beverages, bakery	Qiu et al., 2022
	<i>Yarrowia lipolytica</i>	Limonene	Overexpression of D,L-limonene synthases with co-expression of rate-limiting enzyme	Glucose as feed, pH 5.7, 28°C, 20% oxygen saturation, fed-batch, 22.8 mg/L of limonene	Flouring in food, cosmetics and pharmaceuticals	Pang et al., 2019
		Lycopene	Optimization of lycopene pathway and recovery of strain auxotrophies	YPD10 medium (1% Bacto yeast extract, 2% Bacto peptone, 10% glucose), fed batch reactor, dissolved oxygen 25%, 30°C, jacket), pH 6.8, 21.1 mg/g DCW of lycopene	Natural food colorant and antioxidant	Schwartz et al., 2017
		Violacein	Overexpression of violacein gene cluster and tryptophan biosynthetic genes	Medium containing glucose, 30 °C, 250 rpm for 120 h, 0.366 g/L of violacein	Natural colorant	Gu et al., 2021

(Jinek et al., 2012). Originally discovered as an adaptive immune system in bacteria and archaea, CRISPR protects against invading genetic elements such as phages (Barrangou et al., 2007).

The CRISPR-Cas 9 system consists of a Cas9 nuclease and a guide RNA (gRNA) that directs Cas9 to a specific location in the genome introducing a double-strand break (P. Yang et al., 2024). The cell's natural repair mechanism can repair this break, facilitating the insertion, deletion or modification of genetic material (Rabaan et al., 2023). In eukaryotes, the DSBs can be repaired through either non-homologous end joining (NHEJ) or homologous recombination (HR) pathways (Ali et al., 2022).

The CRISPR-Cas12 is a single-RNA-guided endonuclease used for precise genome editing and pathogen detection. Unlike Cas9, it does not require tracrRNA and shows higher target affinity, enabling efficient and precise targeting of genomic sequences (Hillary & Ceasar, 2023; Rabaan et al., 2023). Cas12-based platforms have been developed for rapid identification and control of multidrug-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* and *Acinetobacter baumannii* (Y. Li et al., 2022).

Bioinformatics-driven pathway analysis helps identify genes for knockout or enhancement to increase protein yield. Strategies include engineering secretion systems for efficient protein release, modifying enzymes for stability and introducing stress-resistant genes to maintain productivity under industrial conditions (Tong et al., 2023).

Strain optimization fine-tunes genetically engineered microorganisms to maximise efficacy and stability in protein production. Techniques such as Adaptive Laboratory Evolution (ALE) expose strains to selective pressures (e.g., temperature, pH, nutrient availability) over multiple generations to enhance robustness and yield (Sandberg et al., 2019). Codon optimization modifies gene sequences to match host-preferred codons, improving translation efficiency, protein folding and post translational modification without altering amino acid composition (Gustafsson et al., 2004).

Omics technologies enable large-scale analysis of biological systems, providing insights into their structure and function at multiple levels. Advances in genomics, transcriptomics, proteomics, and metabolomics provide comprehensive insight into cellular metabolism, enabling identification of bottlenecks and guiding rational engineering strategies (Dai & Shen, 2022).

3.3. Upstream Fermentation Process

In a precision fermentation upstream process (USP), key steps include preparing the growth medium, developing the inoculum culture, sterilizing the bioreactor and associated equipment, combining the seed culture with

sterile medium and initiating production. Throughout the fermentation, conditions must be closely monitored and controlled to maximise the productivity, followed by harvesting for downstream process (DSP).

3.3.1. Feedstock and media optimization

Optimizing feedstock and media is essential for efficient microbial growth and high product yield. Media formulations typically include carbon and nitrogen sources, minerals, vitamins and growth factors tailored for specific microbial host. In precision fermentation, where microbes are engineered to produce target molecules such as proteins and enzymes, media must support cell growth and stability as well as product expression. Carbon sources like glucose, methanol or glycerol are combined with optimised nitrogen sources (e.g., ammonium salts, amino acids) and trace elements and minerals critical for metabolic pathways. Growth factors and vitamins are often customised to enhance biosynthesis and product stability. Optimizing medium composition and substrate choice not only enhances yield but also reduces costs and environmental impact, making precision fermentation a sustainable solution for converting underutilised resources into valuable compounds (Magana-Ortíz et al., 2018)

The success of precision fermentation depends heavily on the growth characteristics of the microorganisms and the composition of the culture medium. Fungi exhibit rapid growth and therefore can play a pivotal role in efficient production when provided with appropriate nutrients (Lübeck & Lübeck, 2022). Heterotrophic fungi require organic carbon but can synthesise amino acids from inorganic nitrogen sources such as ammonia, ammonium salts, nitrate or urea (Lübeck & Lübeck, 2022). The carbon to nitrogen ratio is critical for balancing biomass growth and product formation, and some strains benefit from alternative sugars like molasses or potato starch.

Alternative carbon sources, including agricultural residues (e.g., lignocellulosic biomass), industrial byproducts (e.g. glycerol), and food waste, offer sustainable feedstock but often require pretreatment to remove inhibitors such as furfural and phenolics (Ramírez Rojas et al., 2022). However, all input materials must meet food-grade standards and comply with relevant regulations. Byproducts can vary widely, and if derived from animals, additional requirements under animal by-product regulations may apply to prevent disease transmission. These considerations are essential for ensuring safety and regulatory compliance in any commercial application. Developing strains tolerant to inhibitory compounds or able to metabolise them have significantly improved the feasibility of using these substrates (Chen et al., 2022).

Beyond basic nutrients, precision fermentation increasingly incorporates recombinant proteins, growth and inhibitory factors to improve microbial performance. Growth factors like fibroblast growth factor (FGF), epidermal growth factor (EGF) and insulin-like growth factor (IGF) stimulate cell proliferation or enhance protein synthesis, while inhibitory molecules such as myostatin inhibitors or bone morphogenetic protein antagonists help suppress undesired pathways (Boukid et al., 2023; Guajardo & Schrebler, 2024; Singh et al., 2022).

3.3.2. Cell bank

Cell banking ensures a consistent, well characterised, high quality microbial source for production over the expected lifetime of the product. It consists of a two-tier system: Master Cell Bank (MCB) and Working Cell Banks (WCBs). The MCB serves as the original source, stored under conditions that preserve genetic and phenotypic stability, while WCBs derived from the MCB are used for production (FDA, 2010).

The process begins with thorough strain characterization to confirm genetic integrity, growth performance and absence of contamination. The selected strain is expanded under controlled conditions, aliquoted and cryopreserved and stored frozen under defined conditions ensuring long-term viability and retention of desired traits (WHO, 2013).

3.3.3. Seed train

Seed train development is a critical step in scaling microbial cultures from small starter volumes to industrial scale bioreactors. The process typically begins with inoculating flasks and progresses through sequential vessels – such as shake flask seed fermenters and small-scale bioreactors- before reaching the main production reactor (Kern et al., 2016). Each stage involves stepwise increases in volume and cell density to ensure robust growth and metabolic activity. During the process, the microbial cells adapt to increasingly larger volumes and more complex environmental conditions (Takors, 2012). Controlled conditions, including nutrient rich media, temperature, pH, aeration, and agitation, are essential throughout the process to sustain exponential growth and preserve stress-induced variability (de Mello et al., 2024).

High Cell Density Cryopreservation (HCDC) can significantly reduce seed train duration compared to traditional vial-based expression (Merck KGaA, 2021). This approach involves storing cells in cryobags at very high densities and volumes, which can be thawed and directly inoculated into the first seed train bioreactor. It also reduces contamination risk through closed processing, improves reproducibility by providing consistent starting conditions and enables centralised expansion with global

distribution to multiple production sites. The cryomedium used must protect cells during freezing and thawing while ensuring rapid recovery with minimal lag phase and stable growth and productivity.

Seed train process should result in the culture that is dense and physiologically stable to withstand transfer into large-scale bioreactors without losing its stability and productivity. Optimizing seed train design reduces lag phases, improves scalability and minimises contamination risks, making it a cornerstone of efficient bioprocessing.

3.3.4. Bioreactor stage

The upstream bioreactor stage scales microbial cultures to industrial volumes for large-scale production of desired compounds. This step transitions from seed train development to full-scale precision fermentation, requiring precise control of process conditions such as agitation, aeration, temperature, pH and nutrient supply to sustain exponential growth and product formation (Palladino et al., 2024).

Temperature is a critical parameter in bioreactor operation as microorganisms have narrow optimal range for growth (Zhong, 2011). Deviation from this range slow growth and reduce product synthesis, while excessive heat can cause cell death and impair protein or metabolite production. Most microorganisms grow best at pH 5-7, but pH fluctuates during fermentation due to substrate consumption and metabolite release. Therefore, maintaining optimal pH is essential for growth, enzyme activity and product synthesis. Mixing ensures nutrient distribution, enhances mass and heat transfer and prevents metabolite buildup. Mixing impacts biological performance such as cell growth, productivity and control of temperature, pH and mass transfer. While small-scale reactors maintain homogeneity better, scale-up leads to poor mixing, concentration and heat gradients and reduced mass transfer. Mixing optimization is critical as agitation cannot be increase without risking cell damage. Oxygen transfer is a major challenge in aerobic fermentation processes because oxygen is sparingly soluble in water. While most nutrients dissolve easily, oxygen is often a limiting factor in growth and productivity.

Submerge fermentation (SF) involves cultivating microorganisms in liquid media within closed bioreactors designed to control key parameters such as dissolved oxygen, pH, temperature and agitation (Fasim et al., 2021; Vaishnav et al., 2018). Monitoring and control enable large scale operations in fermenters up to 100,000 litres. SF is widely used in precision fermentation for soluble products like proteins, though it demands high energy for aeration and cooling. SF offers scalability and precise control of fermentation conditions but can face challenges such as high broth viscosity in fungal cultures, which may limit heat and mass transfer (Gong

et al., 2023). Fed-batch system improves productivity by controlled nutrient addition, while continuous system allows prolonged operation but increases contamination risk.

Solid state fermentation (SSF) cultivates microorganisms grow on solid substrate with minimal water and low energy requirements (Soccol et al., 2017). SSF is used for production of protein rich foods, enzymes, organic acids and bioactive compounds (Manan & Webb, 2017). However, scaling SSF remains challenging due to difficulties in maintaining uniform conditions, requiring development of optimised large-scale bioreactors.

3.4. Downstream Processing

3.4.1. Harvesting, clarification and extraction

Harvesting is the first step in downstream processing (DSP), which purifies and refines products of precision fermentation from the fermentation broth (Augustin et al., 2024). By setting up the purification steps that follow, harvesting plays a key role in ensuring the product quality. In SF, harvesting involves separating cells from broth using centrifugation or microfiltration (Chen et al., 2022). In SSF, harvesting requires mechanical separation followed by washing and filtration (Good Food Institute, 2024). Efficient harvesting is critical for yield, purity and DSP efficiency, minimizing product loss and contamination risks.

Extraction aims to remove target product from harvested biomass or fermentation medium along with impurities and residual media (Augustin et al., 2024). Efficient extraction maximises yield and preserves product functionality. Recovery depends on whether the product is intracellular (requiring cell disruption) or secreted (requiring separation from broth). Intracellular products require cell disruption to release the target biomolecules and common methods include:

- Mechanical methods physically break the cell walls: high pressure homogenization, ball milling (Harrison, 2011)
- Chemical methods use agents to dissolve cell membrane: solvents and detergents, alkali applications (Augustin et al., 2024)
- Enzymatic methods use specific enzymes to break the cell walls: glycosidases, proteases, amidases (Chen et al., 2022)
- Sonication method uses ultrasonic waves that create cavitation bubbles that rupture cell upon their implosion (Hoque & Devi, 2025)

Once separated from biomass, product recovery typically involves clarification, extraction, purification and drying, meeting regulatory purity standards. Purpose of the clarification is to remove suspended materials such as cells, debris and other solids and produce particle-free liquid.

Clarification methods fall into two main categories: sedimentation and filtration (Reed & Mackay, 2001). Sedimentation relies on density differences, but gravity settling is typically too slow. Therefore, centrifuges such as tubular, disc-stack and decanter centrifuges are used to rapidly separate biomass from liquid. Settling can be improved via coagulation and flocculation, which increases particle size and enhance separation efficiency.

Filtration removes solids by passing liquid through a porous medium. Dead-end filtration (e.g., Buchner funnels, cartridge filters) is simple but prone to clogging, whereas crossflow filtration uses tangential flow to reduce fouling and is more suitable for dense broths. Membrane filtration (microfiltration and ultrafiltration) separate components based on pore size and provides finer clarification but is affected by concentration polarization and fouling, requiring careful control of operating conditions and regular cleaning. Overall, the choice of method depends on feed properties, desired clarity and scale.

Efficient extraction should maximise the recovered amount of product from fermentation broth or biomass and ensure minimal contamination. Based on different characteristics of the product, different extraction methods are utilised. When the target molecule is soluble in organic solvents, the fermentation broth is mixed with the solvent, allowing selective dissolution of the molecule into organic phase (Q. W. Zhang et al., 2018). The organic phase is then separated and evaporated to recover extracted product.

Aqueous two-phase extraction utilises a system in which the product partitions into one of the phases, typically aqueous, minimizing denaturation or activity loss (Segaran & Chua, 2024). Affinity Extraction relies on specific ligand - target molecule interactions achieving high selectivity. This method is commonly used for biomolecules with a known affinity towards particular ligands such as ions or antibodies.

3.4.2. Separation and purification

Purification processes aim to remove impurities and concentrate the product to meet quality standards for food and regulatory requirements. Precipitation is used in bioprocessing to recover fermentation products by reducing solubility and forming insoluble particles. It tends to be applied earlier in the DSP to concentrate products and reduce liquid volume, though it offers only partial purification. The solubility is reduced via

changes in pH, temperature, ionic strength or by addition of salts, solvents or polymers, causing it to precipitate in the form of insoluble particles (Doran, 2013). Precipitation is followed by filtration or centrifugation to recover precipitated solids.

Salting out method is using high salt concentrations to promote aggregation and precipitations of proteins due to disruption of the hydration barriers between protein molecules. In isoelectric precipitation, adjusting pH to the protein's isoelectric point minimises electrostatic repulsion, which allows protein to precipitate in the solution with low ionic strength. Oppositely charged protein groups experience stronger attractive forces in the presence of organic solvents than in water, leading to protein aggregation. Addition of polymers such as polyethylene glycol cause precipitation while also stabilising the protein.

Selective precipitation aims to separate different proteins from each other resulting in high purity homogenous protein products. Selective precipitation may use ligands capable of selective and reversible binding with particular proteins (affinity precipitation), protein binding dyes or heavy metal ions.

Chromatography is a highly selective technique used for separating and purifying precision fermentation products. It separates molecules between stationary and mobile phase based on differences in chemical properties such as size, charge, hydrophobicity or ligand affinity. It is widely used to achieve high-purity of the proteins, enzymes, and other biomolecules. However, this method is often costly due to the cost of specialised resins and the need of precise process control. Different types of chromatography used in product purification:

- Column chromatography work by passing a mixture through a column packed with a solid adsorbent. The components of the mixture interact differently with the stationary phase, causing them to move at different speed and separate (Das & Dasgupta, 1998).
- Ion-exchange chromatography separates molecules based on their electrical charge. The stationary phase consists of a resin that carries charged groups attracting and holding oppositely charged molecules. By gradually changing the composition of mobile phase the molecules are released in a controlled manner (Cummins et al., 2017).

- Size-exclusion chromatography separates molecules based on their size. The stationary phase contains porous beads that allow smaller molecules to enter the pores, slowing their movement, while larger molecules pass through faster (Coskun, 2016).
- Affinity chromatography relies on specific interactions between a molecule and a ligand attached to the stationary phase. After unbound substances are washed out of the column, the target molecule is eluted by changing conditions such as pH or ionic strength (Firer, 2001).
- High-performance liquid chromatography (HPLC) uses high-pressure pumps to force a liquid mobile phase through a column packed with fine particles achieving fast and high-resolution separation. Different modes of HPLC, such as reverse-phase or ion-pair, allow to analysis of a wide range of compounds (Bonfatti et al., 2008; Coskun, 2016)

Additional purification steps, such as diafiltration and ultrafiltration, may be required for buffer exchange and concentration. The final step in DSP involves converting the purified products into a stable form for long-term storage and transport ensuring its quality and functionality. Different drying techniques such as air, vacuum, freeze or spray drying may be used to remove liquids and preserve the activity of the product. Once dried, different preservation techniques can be applied to ensure long-term shelf life. This may include use of preservatives, pH adjustment or use of inert atmosphere packaging. Proper storage conditions, such as humidity, temperature and use of proper packaging are essential to maintain product stability.

Potential food safety hazards and concerns of precision fermentation

Precision fermentation has emerged as a promising and potentially more sustainable alternative to conventional agriculture and animal-derived products. However, as with any novel biotechnological approach, precision fermentation introduces risks and hazards that must be thoroughly understood, managed and proportionally regulated. The risks associated with precision fermentation manufacturing process fall into several categories: chemical hazards, microbial hazards, hazards associated with the use of genetically modified microorganism and products derived from the precision fermentation.

4.1. Chemical Hazards

4.1.1. Contaminants from raw materials or media

Although precision fermentation uses nutrient broths rather than whole plant matter, chemical hazards can arise from contaminated raw materials. Growth media components may contain trace heavy metals, pesticide residues or organic pollutants if non-grade substrates are used (Balali-Mood et al., 2021; Verdadero et al., 2025). When plant-derived hydrolysates or waste feedstock (e.g., lignocellulosic biomass) is used as source of carbon or nutrients, there is potential to carry-over natural plant toxins such as glycosides, alkaloids or phytoestrogens, as well as anthropogenic contaminants (Jin et al., 2025).

Additional hazards include inducer chemicals used in recombinant production. For example, methanol, commonly employed to trigger gene expression in *Komagataella phaffii*, is toxic and must be removed during downstream processing (Lv & Cai, 2025). Furthermore, antibiotics added to suppress microbial contamination or maintain genetic traits pose risks if residues remain in the final product.

Effective management of these risks require stringent raw material quality control, validated removal of toxic inducers and antibiotics and monitoring for residual contaminants throughout the DSP.

4.1.2. Processing-related chemical contaminants

At the end of the precision fermentation phase, microbial cells or fermentation broth are harvested and processed to extract, separate and purify desired products. Although these steps aim to remove unwanted substances, chemical hazards can arise during DSP.

Residual chemicals from processing are a significant concern. Solvents, detergents, salts, surfactants and antifoam agents used during extraction or purification may remain in the final product if removal is insufficient. Unwanted chemical by-products can also be formed during processing. Heat treatment and pH adjustment may cause denaturation, degradation or chemical reactions between target molecules and other components. Maillard-type reactions, which involve cross-linking of sugars and proteins, are well documented in food processing and can lead to toxic compounds or reduce protein digestibility (Aljahdali & Carbonero, 2017; Qi et al., 2025). Similarly, lipid oxidation during processing may produce harmful compounds that affect food quality and human health (Alizadeh et al., 2025). Chemical reactions during DSP can generate undesirable compounds such as heterocyclic amines or oxidation products which are recognised in conventional food manufacturing.

Media and additive-related hazards also require attention. Chemicals used for pH control, sterilization or extraction, such as acids, bases and solvents can pose risks if residues remain in the final product. Improper handling or disposal of chemical waste spent media or by-products can lead to soil or water contamination.

4.1.3. Chemical hazards related to storage

Products of precision fermentation are purified and formulated before distribution, but as for many foods, their long-term stability is influenced by several chemical degradation pathways. Oxidation is common degradation pathway affecting products, especially those containing lipids or redox-active amino acids. Exposure to oxygen, light or trace metals can initiate oxidative reaction leading to loss of bioactivity, colour change, off flavour or formation of by-products. Many biomolecules are susceptible to hydrolysis, especially under high humidity or in aqueous formulations, leading to decomposition of the product (Dhondale et al., 2023; Towns, 1995). Protein cross-linking, the Maillard reaction, lipid oxidation, lipolysis, and proteolysis may occur during storage of precision fermentation products as it was shown for milk protein concentrates, changing its chemical and physical properties (Fan et al., 2018). Additionally, packaging material can influence stability and be source of chemical contamination as chemicals or allergens may migrate from plastic packaging into food.

4.2. Microbial hazards in precision fermentation

Precision fermentation offers a highly controlled environment for producing proteins, enzymes and other bioactive compounds. However, despite advanced bioprocessing technologies, microbial hazards remain a critical concern throughout USP and DSP operations. These hazards can compromise product safety, quality and regulatory compliance if not properly controlled.

4.2.1. Microbial contamination in UPS

Precision fermentation relies on nutrient-rich media that provide an ideal environment for microbial growth. If aseptic techniques fail or sterility is compromised, contaminating non-target organisms such as bacteria, fungi or viruses can proliferate rapidly (Niyigaba et al., 2025). These contaminants may compete with the targeted genetically modified microorganism used in the precision fermentation, leading to reduced yield, product spoilage or changes in fermentation kinetics. Importantly, spoilage can also serve as a marker for potential microbial food safety.

Spore forming microorganism pose particular challenges because they can survive sterilization and germinate under favourable conditions later in the process (Bressuire-Isoard et al., 2018).

Contamination risks are highest during early fermentation stages when production strain densities are low and during scale-up phases when biomass is transferred between vessels. Precision fermentation relies heavily on the precise control of process conditions such as temperature, pH, oxygen concentration and nutrient availability. Deviation from optimal conditions can lead to reduced efficacy, contamination or the production of the unwanted by-products.

The use of antibiotics to suppress contamination is generally discouraged due to regulatory restrictions, cost implications and concerns regarding antimicrobial resistance. Instead, best practices emphasise rigorous sterility validation, controlled raw materials inputs and continuous monitoring of bioreactor conditions.

4.2.2 Microbial contamination in DSP

After fermentation, microbial cells or fermentation broth undergo downstream processing to isolate the target product. Despite purification steps, microbial hazards can persist. Host cell proteins, lipopolysaccharides, antimicrobial compounds and secondary metabolite may remain in the final product if purification is insufficient. The nucleic acid content of microbial biomass may also pose a risk. High levels of nucleic acids can be harmful to human health, as high levels of purine are toxic (Sturme et al., 2025).

Environmental contamination during processing is another risk. Microbial contamination from the production environment can occur during downstream processing. Strict hygiene practices and continuous monitoring are essential to minimise risk. Large volumes of microbial biomass and spent media generated during processing may contain recombinant biomolecules or non-native compounds. These waste streams require disposal or recycling to prevent contamination and environmental impact.

4.3. Hazards associated with Genetically Modified Microorganisms (GMMS)

Precision fermentation uses genetically modified microorganism as cell factories to produce food ingredients such as proteins, enzymes and other biomolecules under controlled conditions. Unlike traditional fermentation, the final products are purified from microbial biomass or fermentation broth, reducing risk associated with microbial contamination or unwanted metabolites. In accordance with regulatory guidance applicable in GB,

genetically modified microorganisms (GMMs) should not be present in the final product following purification, as legislation requires their removal to ensure product safety and compliance. However, their use raises safety concerns and requires regulatory oversight (Augustin et al., 2024; EFSA, 2011).

4.3.1. Production of toxins and secondary metabolites

Certain microbial hosts used in precision fermentation can produce toxins or secondary metabolites under stress or uncontrolled conditions. Filamentous fungi such as *Aspergillus spp.* or *Trichoderma reesei* may have genetic capacity for mycotoxin production, while some bacterial hosts, including *Streptomyces*, *Enterococcus faecium* and *Clostridium butyricum* are known to synthesise harmful compounds (Augustin et al., 2024; EFSA, 2011).

Currently, only a limited amount of well-studied microbial hosts is used in precision fermentation, despite the theoretical suitability of many species (Pereira et al., 2025). Regulatory frameworks such as the Qualified Presumption of Safety (QPS) in the EU and Generally Recognised as Safe (GRAS) status in the USA support the safe use in food production (EFSA, 2024; FDA, 2016). Microorganisms recognised as QPS or GRAS have a documented history of safe use in food and have undergone rigorous safety evaluations, reducing uncertainty regarding pathogenicity, toxin production or allergenicity. This can accelerate the approval process compared to novel or uncharacterised strains. To meet the growing demand as well as reduce environmental burden of traditional agricultural practices, the industry is exploring use of species with diverse metabolic capacities, but without history of safe use in food industry requiring thorough characterization and assessment of potential risks associated with these species.

4.3.2 Horizontal Gene Transfer

Horizontal gene transfer (HGT) is a fundamental biological process that enables movement of genetic material between organisms outside of vertical inheritance. While the probability of the transfer varies, the consequences can include ecological disruption, persistence of engineered traits and public health risk. HGT occurs primarily through transformation, conjugation and transduction (Emamalipour et al., 2020). This could result in the spread of novel traits such as metabolic functions, production of bioactive compounds or transferal of antimicrobial resistance genes into wild microbial communities with unpredictable ecological consequences.

4.4. Potential safety concerns of precision fermentation derived molecules

Precision fermentation can produce proteins or other molecules that are novel to the human diet, mimic animal-derived molecules (e.g., dairy or egg proteins, meat flavour), or represent entirely new compounds such as bioactive peptides, enzymes and speciality lipids.

Novel proteins may trigger allergic responses, particularly, when the target protein mimics a common allergen like milk or egg. Proteins of animal origin have often different post-translation modifications compared to those produced by microorganisms, which may impact the allergenicity of the protein (Halim et al., 2015; Jin et al., 2025). To date, no evidence suggests that proteins produced via precision fermentation differ substantially in allergenicity from their animal-derived counterparts. However, given the diversity of proteins that can be produced and the potential influence of host-specific post-translational modifications (PTMs), further investigation into their impact on allergenicity is warranted (Jin et al., 2025). Precision fermentation products will differ depending on factors such as specific gene used for the protein expression, microorganism species used in process, culture media and other processing conditions, which may impact the allergenicity of each product (Grundy et al., 2024).

Novel molecules may exhibit unintended biological activity, including enzyme function, receptor binding or metabolic effects. Chronic dietary exposure could lead to unforeseen consequences such as metabolic disruption or immune responses. Long-term safety data for many precision fermentation-derived compounds is limited. Precision fermentation derived proteins and peptides may influence gut microbial composition, promoting or suppressing certain species and generating bioactive metabolites (Peng et al., 2024). Replacing animal-derived ingredients with alternatives can alter amino acid profiles, digestibility and micronutrient content. Improper formulation may lead to nutritional imbalances.

5. Conclusion

Precision fermentation represents a promising approach to address sustainability and nutritional challenges in the global food system. By employing genetically modified microorganisms, precision fermentation enables production of high-value compounds such as proteins, enzymes and other food ingredients traditionally sourced from animal or plants. While precision fermentation does not introduce fundamentally new hazards compared to conventional fermentation or other biotechnologies, the novelty of engineered hosts and structurally distinct products requires robust, multi-layered risk assessment. These assessments are complex

because they must integrate chemical, microbial, genetic and process-related factors while addressing regulatory and consumer safety requirements.

Why Safety Assessment is Complex

- **Genetically Modified Microorganisms (GMMs)**
Their use introduces regulatory and ecological considerations, including complete removal from final products and prevention of horizontal gene transfer to environmental microbes.
- **Novel Proteins and Analogues**
Proteins produced via precision fermentation may differ in post-translational modifications from animal-derived counterparts, raising potential allergenicity or unintended bioactivity concerns.
- **Chemical Hazards**
Residual solvents, detergents, antifoam agents, and inducers (e.g., methanol) from upstream and downstream processing must be controlled. Feedstocks, especially waste-derived substrates, may introduce heavy metals, pesticides, or natural toxins.
- **Microbial Hazards**
Nutrient-rich media and scale-up steps create contamination risks. Persistence of host cell proteins, nucleic acids, or secondary metabolites in purified products can compromise safety.
- **Process-Related Risks**
Scale-up challenges—oxygen transfer, mixing, pH control—can induce microbial stress responses, triggering toxin or metabolite production.
- **Environmental and Waste Management**
Disposal of spent media and biomass containing recombinant material requires strict containment to prevent contamination and ecological impact.

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