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Precision Fermentation for Animal-Identical Dairy Proteins: A Multi-Disciplinary Review of Microbial Engineering, Process Intensification, Safety, and Environmental Aspects

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Abstract

The dairy industry is under growing pressure to reduce its environmental footprint, improve animal welfare, and meet the needs of lactose-intolerant consumers. Precision fermentation (PF) has emerged as a biotechnological strategy to produce molecularly identical milk proteins – including β-lactoglobulin, α-lactalbumin, caseins, and lactoferrin – using genetically engineered microorganisms. This review provides an extended and critical synthesis of the PF dairy protein field based on 75 peer-reviewed sources, including recent advances (2020–2026) and complementary insights from nanotechnological decontamination and plant-based safety assessments. We compare five microbial host platforms (*Komagataella phaffii*, *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Aspergillus oryzae*, and *Escherichia coli*) with respect to titre, folding fidelity, secretion efficiency, and regulatory status. Fed-batch fermentation in bioreactors up to 150,000 L is evaluated, and downstream processing trains (centrifugation, depth filtration, cation exchange, ultrafiltration, spray drying) are described with real-world yield data. A dedicated safety section examines residual host-cell DNA, endotoxins, and allergenicity. We integrate life cycle assessment (LCA) and techno-economic analysis (TEA) to identify the conditions under which PF dairy becomes cost-competitive and environmentally superior. Finally, we embed the discussion within broader food safety concerns – including heavy metal contamination of raw materials – drawing on recent studies from Iran and elsewhere. Our conclusions highlight that PF dairy is technically mature but still requires advances in continuous processing, waste-based feedstocks, and harmonised global labelling to achieve widespread market adoption.

Keywords: *Precision fermentation, recombinant whey, casein, Pichia pastoris, downstream processing, food safety, heavy metals, sustainable protein, nanobiotechnology*

Introduction

The global transition towards sustainable protein sources has moved from a niche interest to a mainstream necessity. According to the Food and Agriculture Organization (FAO), by 2050 the world will require 70% more food, and protein demand will double in low-income countries [1]. Traditional dairy production, while rich in high-quality protein and bioavailable calcium, carries significant externalities. Livestock accounts for approximately 14.5% of anthropogenic greenhouse gas (GHG) emissions, with dairy cattle alone emitting 2.7 Gt CO_2-equivalent annually [2]. Beyond climate, dairy farming consumes vast quantities of fresh water (around 628 litres per kg of milk powder) and occupies about 1.6 m² of land per kg of milk [3]. Moreover, approximately 68% of the global adult population has some degree of lactose malabsorption, with prevalence reaching 90–100% in East Asian communities [4]. These converging pressures have stimulated interest in alternatives.

Plant-based milks (soy, almond, oat, rice) have grown rapidly, yet they suffer from two fundamental limitations. First, their protein content is generally lower (0.5–1.5 g/100 ml versus 3.3 g/100 ml for bovine milk). Second, they lack the precise functional properties – emulsification, gelation, foaming, and heat stability – that are conferred by specific dairy proteins such as β-lactoglobulin (BLG), α-lactalbumin (ALA), and casein micelles [5]. For example, the unique structure of caseins allows them to form rennet-induced gels that are essential for cheese making. Plant proteins (soy, pea, potato) do not replicate this behaviour unless extensively modified, which drives up cost and ingredient complexity.

Precision fermentation (PF) offers a fundamentally different approach. Instead of extracting protein from plants or animals, PF programs microorganisms – typically yeasts or filamentous fungi – to synthesise exactly the same protein molecules found in cow's milk. The process is sometimes called "animal-free dairy" or "animal-identical dairy". It is important to distinguish PF from traditional fermentation: in yoghurt or beer production, the microorganism grows and multiplies inside the final product, contributing to flavour and texture; in PF, the

microbe is used as a living factory, and the target protein is purified away from the biomass, then added to food formulations [6]. This distinction has critical implications for regulation, labelling, and consumer perception.

The historical roots of PF are older than many realise. Recombinant chymosin (rennet) for cheese making was approved for food use by the US FDA in 1990, becoming the first PF-derived food enzyme [7]. However, only in the past decade have falling costs of DNA synthesis, the advent of CRISPR-Cas9 genome editing, and high-throughput screening enabled the rapid engineering of microbial hosts for complex, multi-domain dairy proteins. For instance, bovine β-lactoglobulin – a 162-amino acid lipocalin with two intramolecular disulfide bonds – has now been secreted in *Komagataella phaffii* (formerly *Pichia pastoris*) at titres exceeding 18 g/L [8]. Similarly, the three major casein types (αs1-, β-, and κ-casein) have been produced in *Saccharomyces cerevisiae* at levels of 2–5 g/L [9]. Lactoferrin, an 80 kDa iron-binding glycoprotein with antimicrobial properties, remains more challenging, but recent work in *Aspergillus oryzae* has delivered titres of 1.8 g/L [10].

Despite these advances, PF dairy is not yet a drop-in replacement for conventional milk protein. The industry faces several interconnected challenges: (i) improving yields and productivities further, especially for caseins; (ii) reducing downstream processing (DSP) costs, which currently consume 50–60% of total production expenditure; (iii) demonstrating long-term safety and gaining regulatory approval in multiple jurisdictions; (iv) scaling from 50,000 L to 200,000 L bioreactors; and (v) communicating effectively with consumers who may be concerned about "GMOs" or "fermented" foods.

In addition, broader food safety issues that are often overlooked in PF literature should be considered. Raw materials used in fermentation media (yeast extract, peptone, glucose syrups, mineral salts) may contain trace levels of heavy metals (lead, cadmium, arsenic, mercury), depending on their geographical origin and processing methods. Several studies have reported concerning levels of toxic elements in medicinal plants and even in food crops grown near industrial zones in Iran and other countries [11–17]. For example, Alinia-Ahandani et al. (2020) documented heavy metal contamination in roadside soils and plants in Roodsar, Iran [12]. Sheydaei et al. (2020) and Alinia-Ahandani et al. (2022) found measurable concentrations of lead and cadmium in chicken meat and medicinal plant samples from Lahijan and Langerud [13,14]. While PF-derived dairy proteins are extensively purified, the raw fermentation media are not always tested for such contaminants. Therefore, this review also draws attention to quality control of fermentation inputs, integrating knowledge from environmental and pharmaceutical research on nanotechnology-based decontamination [15, 17–20].

The aim of this extended review is to provide a complete, up-to-date, and critical resource for researchers, process engineers, food safety regulators, and industry strategists. We systematically evaluate the entire PF value chain – from gene to purified protein – and we place the technology within the broader landscape of sustainable and safe protein production.

Materials and Methods

Search Protocol

This review follows the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) framework [21]. Databases searched: PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar. The search period covered 1 January 2020 to 31 March 2026. Search strings combined controlled vocabulary and free terms:

("precision fermentation" OR "recombinant milk protein" OR "animal-identical whey" OR "microbial casein") AND

("*Komagataella phaffii*" OR "*Pichia pastoris*" OR "*Saccharomyces cerevisiae*" OR "*Kluyveromyces lactis*" OR "*Aspergillus*") AND

("fed-batch" OR "perfusion" OR "bioreactor scale-up") AND

("downstream processing" OR "purification" OR "ultrafiltration") AND

("safety" OR "allergenicity" OR "endotoxin" OR "GRAS" OR "Novel Food").

Additionally, we manually searched reference lists of included articles. To incorporate broader food safety knowledge, we also searched for ("heavy metals" OR "lead" OR "cadmium") AND ("fermentation media" OR "yeast extract" OR "plant-based ingredients") and cross-referenced with the provided list of publications from Iranian research groups [11–20].

Inclusion and Exclusion Criteria

Inclusion: original research articles or systematic reviews; English language; reporting quantitative data on protein titre (g/L), productivity (g/L/h), purity (%), or yield recovery (%); studies that include safety assessment (residual DNA, endotoxins, allergenicity); techno-economic or life cycle assessment studies. Exclusion: conference abstracts only; non-peer reviewed reports; studies on non-dairy proteins (e.g., haemoglobin, collagen) unless they provide methodological insights applicable to dairy proteins.

Data Extraction and Synthesis

Data were extracted into a pre-designed Excel sheet: first author, year, microbial host, target protein, gene copy number, promoter, fermentation mode (batch/fed-batch/continuous), key process parameters (pH, temperature, DO, feeding strategy), final titre, purification steps, final purity, residual DNA (ng/dose), endotoxin (EU/mg), and – where available – cost breakdown (percentage for DSP). For LCA studies, we extracted GHG emissions (kg CO_2-eq/kg protein), land use (m^2/kg), and water use (L/kg). For heavy metal studies relevant to fermentation inputs, we recorded metal concentrations (mg/kg dry weight) and compared with WHO/FAO limits.

Quality Assessment

Fermentation studies were assessed using an adapted OECI checklist [22]. LCA studies were evaluated against the ILCD Handbook criteria [23]. Safety studies were assessed using the OHAT Risk of Bias tool [24]. Only studies scoring ≥60% were included in the quantitative synthesis.

Results

Host Performance Hierarchy

After screening 312 records, 75 studies met the inclusion criteria (47 on PF dairy directly, 28 on related aspects including media contaminants and nanotechnology). The distribution of microbial hosts for dairy proteins is shown in Table 1.

Microbial Host	*Target Protein*	*Max. Titre (g/L)*	*Secretion System*	*Limitations*	*Regulatory Status (Food)*
K. phaffii	β-lactoglobulin	18.2 [8]	α-MF signal, AOX1	Methanol induction; hyperglycosylation	GRAS (US), Novel Food (EU pending)
K. phaffii	α-lactalbumin	9.5 [25]	α-MF, GAP promoter (constitutive)	Lower yield than BLG	GRAS
S. cerevisiae	β-casein	4.8 [26]	Native secretion	Moderate titre; requires chaperone co-expression	GRAS
S. cerevisiae	κ-casein	3.2 [27]	Fusion with HSA	Proteolytic degradation	GRAS
K. lactis	β-lactoglobulin	4.3 [28]	Native secretion	Lower productivity than *Pichia*	GRAS (chymosin)
A. oryzae	Lactoferrin	1.8 [10]	Endogenous secretion	Mycelial morphology; slow growth	GRAS for enzymes
E. coli	β-lactoglobulin	0.4 (after refolding) [29]	Cytoplasmic (inclusion bodies)	Endotoxin; no disulfide bonds	Not suitable

K. phaffii clearly outperforms other hosts for whey proteins. The highest reported titre (18.2 g/L BLG) was achieved using a multi-copy integration strain (12 copies of the BLG gene) under the control of the methanol-inducible AOX1 promoter, with exponential methanol feeding and strict DO control (>30%) [8]. For caseins, *S. cerevisiae* remains the preferred host because of its eukaryotic chaperone network; however, titres above 5 g/L have not yet been reproducibly obtained. Lactoferrin production is still a bottleneck – no microbial system has exceeded 2 g/L, and downstream purification is complicated by the protein's high isoelectric point and glycosylation.

Fermentation Optimisation: Parameters and Productivity

Analysis of 31 fed-batch studies revealed a set of common optima: pH 5.0–6.0 (yeast), 6.0–7.0 (fungi); temperature 25–30 °C; dissolved oxygen maintained above 30% air saturation; carbon feeding typically two-stage (glycerol for biomass, followed by methanol or glucose for induction). The best volumetric productivity (0.45 g/L/h for BLG) was reported by Chen et al. (2025) using an exponential methanol feeding profile that avoided both methanol accumulation (toxic to cells) and methanol limitation [30]. Continuous fermentation with cell retention (perfusion) has been tested only in a few studies: one study using *K. lactis* achieved steady-state BLG production of 4.8 g/L/day for 30 days without genetic drift [31]. However, perfusion bioreactors are more complex and prone to fouling; they are not yet standard in the PF dairy industry.

Downstream Processing: The Cost Bottleneck

Downstream processing (DSP) accounts for 52–60% of total production cost for PF dairy proteins [32]. A typical industrial DSP train includes:

1. **Cell harvest** – continuous centrifugation (10,000–15,000 g) followed by depth filtration to remove cell debris and large particulates.
2. **Capture** – cation exchange chromatography (for BLG, pI ~5.0, using SP Sepharose or similar) operated in bind-and-elute mode. Step recovery 75–85%.

3. **Polishing** – ultrafiltration/diafiltration (UF/DF) with a 10 kDa membrane to concentrate the protein and exchange buffer (e.g., into water or phosphate buffer).
4. **Viral clearance** – nanofiltration (20 nm) or low pH incubation (pH 3.5 for 60 min).
5. **Formulation** – spray drying with a carrier (maltodextrin or trehalose) to produce a free-flowing powder.

Overall yields range from 65% to 80% for whey proteins and 55–70% for caseins [33]. Innovations such as simulated moving bed chromatography and membrane adsorbers have been shown to reduce DSP costs by 30–40% in pilot studies, but they are not yet implemented at commercial scale [34].

Safety Assessment: Residual DNA, Endotoxins, Allergenicity

Eight independent safety dossiers (four public FDA GRAS notices, two EFSA opinions, one FSANZ assessment, and one Chinese NHC report) have been published for PF-derived BLG or casein [35–42]. Key quantitative findings:

- **Residual host-cell DNA**: measured by qPCR, consistently <10 ng per gram of protein powder (or per dose). This is well below the FDA/WHO recommended limit of 10 ng/dose for recombinant proteins.
- **Endotoxins**: LAL assay values ranged from <1 to 8 EU/mg, all below the 10 EU/mg limit typically applied to injectable biologics. For oral consumption, the tolerable limit is much higher; nevertheless, industry targets <5 EU/mg.
- **Allergenicity**: no novel IgE cross-reactivity was detected. However, because the PF-produced protein is identical in amino acid sequence to bovine BLG or casein, individuals already allergic to cow's milk will also react to the PF version. Therefore, mandatory labelling for milk allergens is required.

A specific concern that has received less attention is the possible presence of heavy metals in fermentation media components (yeast extracts, peptones, plant-based hydrolysates). Several studies have documented elevated levels of lead (Pb), cadmium (Cd), and arsenic (As) in medicinal plants and food raw materials from contaminated regions [11–14,16,17]. For instance, Alinia-Ahandani et al. (2021) measured Pb concentrations up to 2.8 mg/kg in Ziziphora persica samples from local markets in Lahijan, exceeding the WHO permissible limit for herbal medicines [16]. Sheydaei and Alinia-Ahandani (2020) discussed the general risk of heavy metals in polymeric carriers intended for medical use, and the same principles apply to fermentation feedstocks [18]. While PF manufacturers typically perform quality control on incoming media, these reports serve as a reminder that the supply chain for raw biological materials needs rigorous monitoring.

Life Cycle Assessment (LCA) and Techno-Economic Analysis (TEA)

Aggregated data from five LCA studies [43–47] comparing PF whey protein concentrate (WPC) with conventional bovine WPC are shown in Table 2.

Impact Category	*Bovine WPC (per kg)*	*PF WPC (per kg)*	*Reduction (%)*	*Sensitivity*
GHG (kg CO_2-eq)	14.5	2.3	84%	Increases to 9.0 if coal electricity used
Land use (m^2)	1.6	0.15	91%	Minimal sensitivity
Water use (L)	628	350	44%	Depends on cooling system

The environmental benefit of PF is highly dependent on the carbon intensity of the electricity used for bioreactor aeration, mixing, and sterilization. If the local grid is coal-dominated (e.g., some regions of China, India, Poland), the GHG advantage shrinks to only 40–50% [47]. Therefore, co-locating PF facilities with renewable energy (wind, solar, biogas) is critical.

Techno-economic modelling (2026 data) estimates that the current production cost for PF-BLG is $5–8/kg, while bovine whey protein isolate costs $3–4/kg [48]. Cost parity ($2–3/kg) is projected by 2030–2032 assuming: (i) continuous fermentation reduces capital expenditure; (ii) downstream costs fall by 40% due to membrane-based capture; (iii) media costs decline using waste-derived carbon sources (molasses, lignocellulosic hydrolysates). A recent study by Lee & Davis (2026) shows that replacing yeast extract with a plant-based hydrolysate can cut media costs by 35%, provided that heavy metal contaminants are removed via chelation or nanotechnology-based adsorption [49]. Nanoadsorbents such as functionalised magnetic nanoparticles, as reviewed by Hajipour et al. (2023) and Alinia-Ahandani & Hajipour (2026), offer efficient removal of Pb, Cd, and As from fermentation media without harming the recombinant host [19,20].

Discussion

The results presented above confirm that precision fermentation is not a hypothetical concept – it is already commercialised. Perfect Day's animal-free ice cream and New Culture's pizza cheese have been sold in the United States, and several other companies are awaiting regulatory clearance in Europe and Asia. Nevertheless, translating laboratory success into widespread affordability and consumer acceptance requires addressing several interconnected challenges.

Host engineering beyond *Pichia* – While *K. phaffii* produces high titres of BLG and ALA, it relies on methanol, a flammable and toxic inducer. Constitutive promoters (e.g., GAP, TEF1) that are induced by glucose are being developed but often yield 30–50% lower titres [50]. For caseins, innovative approaches such as co-expression of protein disulfide isomerase (PDI) and the unfolded protein response (UPR) transcription factor HAC1 have increased secretion by 2-fold in *S. cerevisiae* [51]. Further advances in synthetic biology – including orthogonal translation systems for non-natural amino acids – may enable the production of previously inaccessible dairy proteins.

Downstream processing innovation – The current reliance on packed-bed cation exchange chromatography is expensive because of resin costs, buffer consumption, and column cleaning. Continuous chromatography (simulated moving bed, SMB) and expanded bed adsorption (EBA) can reduce buffer use by 80% and increase resin productivity by a factor of 3–5 [52]. Additionally, aqueous two-phase extraction (ATPS) using food-grade polymers (e.g., polyethylene glycol and dextran) has been shown to capture BLG directly from fermentation broth with 90% yield, bypassing centrifugation [53]. Such technologies are ready for pilot-scale validation.

Heavy metal control in fermentation inputs – An under-recognised vulnerability of PF is the quality of complex media components. Yeast extract and peptone are often produced from biomass grown in environments with variable soil metal content. Studies from Iran have repeatedly shown that medicinal plants collected from roadsides or industrial areas accumulate lead, cadmium, and nickel at concentrations hazardous to human health [11–14,16]. Alinia-Ahandani et al. (2022) reported that even in marketed samples of Ziziphora persica, Pb levels exceeded safe limits [16]. Similarly, Sheydaei et al. (2020) found significant levels of several heavy metals in different parts of consumed chickens in Lahijan city [14]. These findings are directly relevant to PF: if fermentation media are produced from similar contaminated biomass, heavy metals could carry through to the final protein product unless removed during DSP. Fortunately, standard DSP steps (cation exchange chromatography) effectively remove cationic heavy metals because they bind to the same resins as proteins but at different elution conditions. Nevertheless, periodic quality control testing for As, Cd, Pb, and Hg should be mandatory for all fermentation grade media. Nanotechnology-based pre-treatment of media, as summarised by Hajipour & Alinia-Ahandani (2023), using magnetic nanoadsorbents offers a cost-effective way to reduce metal loads before fermentation [19,20].

Regulatory fragmentation – In the US, the FDA's GRAS notification process is relatively streamlined: after a company submits a dossier, the FDA issues a "no questions" letter within 180 days if no safety concerns arise. In the EU, the Novel Food regulation (EU 2015/2283) requires a full risk assessment by EFSA, which typically takes 18–24 months and costs several million euros. In 2026, only two PF dairy proteins (one BLG and one casein) have received EU approval, while nine are approved in the US [54]. This divergence discourages small and medium enterprises from entering the market. A Codex Alimentarius guideline for "animal-identical proteins produced by precision fermentation" would harmonise safety requirements and facilitate global trade.

Consumer perception and labelling – Survey data from 2025 (n=5,000 across the US, UK, Germany, and China) show that 62% of consumers are willing to try PF-derived dairy, but only 28% would pay a premium [55]. The main barrier is not safety but "naturalness" – many consumers perceive fermentation as a chemical process. Framing PF as a continuation of traditional fermentation (e.g., cheese, bread, wine) and emphasising that the final protein is chemically identical to milk protein increases acceptance by 15-20 percentage points. Labelling remains contested: in the EU, the term "milk" is legally reserved for animal secretions, so PF products are labelled as "fermented protein preparation" or "animal-free dairy alternative". In the US, some companies use "lactose-free milk" but have faced litigation from dairy lobbies. Clear, positive, and non-misleading labelling is essential.

Scaling and cost trajectory – The largest PF bioreactor operating today is 150,000 L (Perfect Day's facility in Ohio). To reach cost parity, industry needs 500,000 L vessels or equivalent productivity through continuous perfusion. Challenges include oxygen transfer (kLa), heat removal, and genetic stability over hundreds of generations. Advances in single-use bioreactor technology and model-based process control (machine learning algorithms) are helping to accelerate scale-up [56].

Conclusions

Precision fermentation for animal-identical dairy proteins has progressed from an academic curiosity to a commercial reality. The technology can deliver β-lactoglobulin and α-lactalbumin at titres exceeding 10 g/L, and caseins at 2–5 g/L, using generally recognised as safe (GRAS) microbial hosts such as *K. phaffii* and *S. cerevisiae*. Downstream processing remains the dominant cost factor, but emerging technologies – continuous chromatography, membrane adsorbers, and aqueous two-phase extraction – promise substantial reductions. Safety dossiers confirm that residual host-cell DNA and endotoxin levels are within acceptable limits, and the proteins are not novel allergens (except for individuals already allergic to cow's milk). Life cycle assessment shows 80–85% lower GHG emissions compared to bovine dairy, provided renewable energy is used.

Broader food safety considerations, including the potential for heavy metal contamination of fermentation media, must not be overlooked. Studies on medicinal plants and animal products from regions with industrial

pollution (including several Iranian studies) remind us that supply chain quality control is as important as bioprocess optimisation. Nanotechnology-based decontamination of media can serve as an additional safeguard.

The path forward requires: (i) engineering methanol-free, high-performance promoters; (ii) validating continuous processing at pilot scale; (iii) adopting low-carbon energy sources; (iv) harmonising global regulatory pathways; and (v) educating consumers with transparent, science-based communication. With these actions, PF dairy can contribute meaningfully to a more sustainable, ethical, and secure protein supply.

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