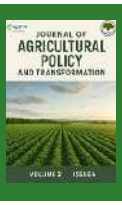


Journal of Agricultural Policy and Transformation *(AgriPaT)*
ISSN: 3134-819X
Journal Homepage: http://www.ageconfrontiers.com/agripat
Vol. 2(4) Open Access

Lactase in Dairy Processing: Current Advances, Challenges, and Future Directions — A PRISMA-Informed Narrative Review

Ali Raza[1*], Lu Xiaodong[1], Adnan Amjad[2], Faizan ul Haq[3] & Zhouping Wang[1]
[1]School of Food Science and Technology, Jiangnan University, Wuxi, 214122, P.R. China
[2] Faculty of Food Science and Nutrition, Bahauddin Zakariya University, Multan, Pakistan
[3] Yangzhou University, Yangzhou, Jiangsu, 225127, P.R. China
[*] *Corresponding author: razi.bwn@gmail.com*

ARTICLE INFO
Keywords
- *Lactase*
- *β-galactosidase*
- *Lactose intolerance*
- *Lactose-free dairy products*
- *Enzyme immobilization*
- *Protein engineering*
- *Whey valorization*
- *Food regulation*

Article History
Received: 01 August 2026
Revised: 03 September 2026
Accepted: 03 September 2026
Available online: 03 September 2026

DOI
10.66529/agripat.2026.2.4.121

ABSTRACT
Lactase (β-galactosidase, EC 3.2.1.23) hydrolyses lactose into glucose and galactose and plays a central role in the production of lactose-free and low-lactose dairy products. This review synthesizes peer-reviewed literature published mainly between 2018 and 2026 on lactase sources, mechanisms, industrial applications, limitations, and emerging engineering strategies. Lactase-mediated hydrolysis supports the production of lactose-free milk, yogurt, cheese, ice cream, whey protein powders, and infant formulas while largely preserving nutritional and sensory quality. However, wider industrial application remains constrained by enzyme sensitivity to temperature and pH, high production and purification costs, and inconsistent regulatory thresholds for lactose-free and low-lactose labelling. Recent advances in protein engineering, directed evolution, and enzyme immobilization, including metal-organic frameworks, electrospun nanofibers, and agro-industrial waste-derived supports, have improved thermostability, reusability, and resistance to product inhibition. Combining lactase treatment with ultrafiltration, microencapsulation, and high-pressure processing, alongside whey valorization, offers opportunities for more sustainable and cost-effective production. Key research gaps include limited techno-economic and life-cycle assessments, insufficient consumer-acceptance evidence from underrepresented regions, and the early-stage development of personalized lactase-nutrition approaches. Interdisciplinary research integrating enzymology, process engineering, regulatory science, and consumer research is therefore needed to advance lactase-enabled dairy products globally.

Citation: *Raza, A., Xiaodong, L., Amjad, A., Haq, F.U. & Wang, Z. (2026). Lactase in Dairy Processing: Current Advances, Challenges, and Future Directions — A PRISMA-Informed Narrative Review, J. Agric.* Pol. & Transf. 2(4): 224-233.
https://doi.org/10.66529/agripat.2026.2.4.121

1. INTRODUCTION

Lactase, also known as β-galactosidase (EC 3.2.1.23), catalyzes the hydrolysis of lactose, the principal disaccharide in mammalian milk, into its constituent monosaccharides, glucose and galactose. In the human small intestine, this reaction is normally carried out by lactase-phlorizin hydrolase anchored in the brush-border membrane of enterocytes. Following weaning, most mammals, including a majority of humans, undergo a genetically programmed decline in intestinal lactase expression, a phenomenon termed lactase non-persistence (Novalin et al., 2005; Swagerty et al., 2002). When lactase activity falls below the level required to digest dietary lactose, unabsorbed lactose is osmotically active and is fermented by colonic microbiota, producing short-chain fatty acids and gas that manifest as bloating, abdominal pain, flatulence, and diarrhea, collectively described as lactose intolerance (Swagerty et al., 2002). Global estimates of lactose malabsorption prevalence vary by methodology and population sampled, but multiple independent sources converge on a range of approximately 65–70% of the adult world population, with marked ethnic and geographic variation: prevalence is lowest among individuals of Northern European ancestry (as

low as 5–15%) and highest among East Asian, Sub-Saharan African, and Indigenous American populations, where it can exceed 90% (Li et al., 2023b; Malik & Panuganti, 2024). This distribution reflects the geographically patchy evolutionary spread of the lactase-persistence allele among pastoralist populations (Swagerty et al., 2002).

The dairy industry has responded to this widespread physiological constraint by using exogenous lactase to hydrolyze lactose during or after processing, yielding lactose-free and low-lactose analogues of milk, yogurt, cheese, ice cream, whey protein isolates, and infant formula (Dominici et al., 2022; Li et al., 2023a). Because hydrolysis converts lactose into the sweeter monosaccharides glucose and galactose, enzymatic treatment simultaneously improves digestibility and sensory sweetness, in some cases reducing the need for added sugar in flavored products (Dekker et al., 2019; Suri et al., 2019). A recent systematic review of gastrointestinal outcomes concluded that lactose-free and low-lactose dairy consumption reduces self-reported gastrointestinal symptoms relative to conventional dairy in lactose-intolerant individuals, while preserving the calcium, vitamin D, and high-quality protein content associated with dairy consumption (Sharp et al., 2021).

Despite this progress, the industrial application of lactase remains constrained by enzyme instability under processing conditions, the cost of enzyme production and formulation, variable consumer acceptance, and a fragmented international regulatory landscape governing “lactose-free” claims (Li et al., 2023a; Taeger & Thiele, 2021). This review provides an updated, critical synthesis of the current status of lactase applications in dairy products, examines these constraints in depth, and evaluates recent biotechnological advances particularly in protein engineering and enzyme immobilization that are beginning to address them. Unlike earlier narrative treatments of this topic, this review explicitly identifies methodological and evidentiary gaps in the literature and proposes a research agenda for closing them.

2. REVIEW METHODOLOGY

Protocol and Review Type

This review follows PRISMA 2020 reporting conventions for search and selection transparency (identification, screening, eligibility, inclusion) to the extent feasible for a single-reviewer, non-registered review. It should be characterized as a narrative review informed by a systematic-style search process rather than a registered PRISMA systematic review or meta-analysis in the strict sense: no protocol was prospectively registered (e.g., in PROSPERO), study selection was not performed by two independent reviewers with a documented adjudication process, and no quantitative effect-size pooling was undertaken. This distinction is disclosed explicitly because PRISMA was designed primarily for systematic reviews of intervention, diagnostic, or epidemiological studies answering a focused clinical question, whereas the present review synthesizes a heterogeneous, cross-disciplinary technology and applications literature. Readers requiring a fully registered systematic review should treat the process below as indicative rather than as a substitute for an independently reproduced, dual-reviewer search.

Consistent with this distinction, Sections 3 through 5 of this review are written as a critical narrative synthesis: they integrate established mechanistic, epidemiological, and regulatory background, drawn from foundational and review-level sources, with specific, quantitative findings drawn directly from the primary studies identified through the search strategy described below. Where a specific numeric result, comparison, or performance outcome is reported (most densely in Sections 6, 7, and 8), it is traceable to a named primary study among the sources included in this review; where a statement instead reflects general, well-established background rather than a single traceable primary finding, this is signaled by citation to a review article or a standard reference work rather than to a primary data source.

Eligibility Criteria

Eligible sources were peer-reviewed primary research articles, peer-reviewed review articles, and recognized clinical/technical reference works (e.g., StatPearls) addressing lactase/β-galactosidase enzymology, dairy processing applications, enzyme engineering or immobilization, lactose intolerance epidemiology, or dairy regulatory standards, published in English. Sources were excluded if they were non-peer-reviewed commercial market reports, blog or trade-association content without primary data, addressed enzymes unrelated to β-galactosidase, or were subsequently retracted. Publication years were not formally restricted, but priority was given to sources from 2018 onward, with earlier foundational or epidemiological sources retained only where still current and uncontradicted by more recent evidence.

Two minor exceptions to this peer-review rule were made and are explicitly disclosed here: first, an unpublished Bachelor's thesis (Meehan, 2021) was retained because it was the only identified source providing directly measured lactose-content data for commercially labelled “lactose-free” whey-protein sports-nutrition products, a specific quality-control gap not otherwise addressed in the peer-reviewed literature; and second, an industry-institute epidemiological report (Malik & Panuganti, 2024) was retained because it synthesizes recent, regionally disaggregated lactose-intolerance prevalence data that are consistent with, and corroborated by, the peer-reviewed

epidemiological sources cited alongside it in Section 1. Both sources are treated as documented, justified exceptions to the general eligibility rule rather than as core evidentiary pillars of the review.

Information Sources and Search Strategy

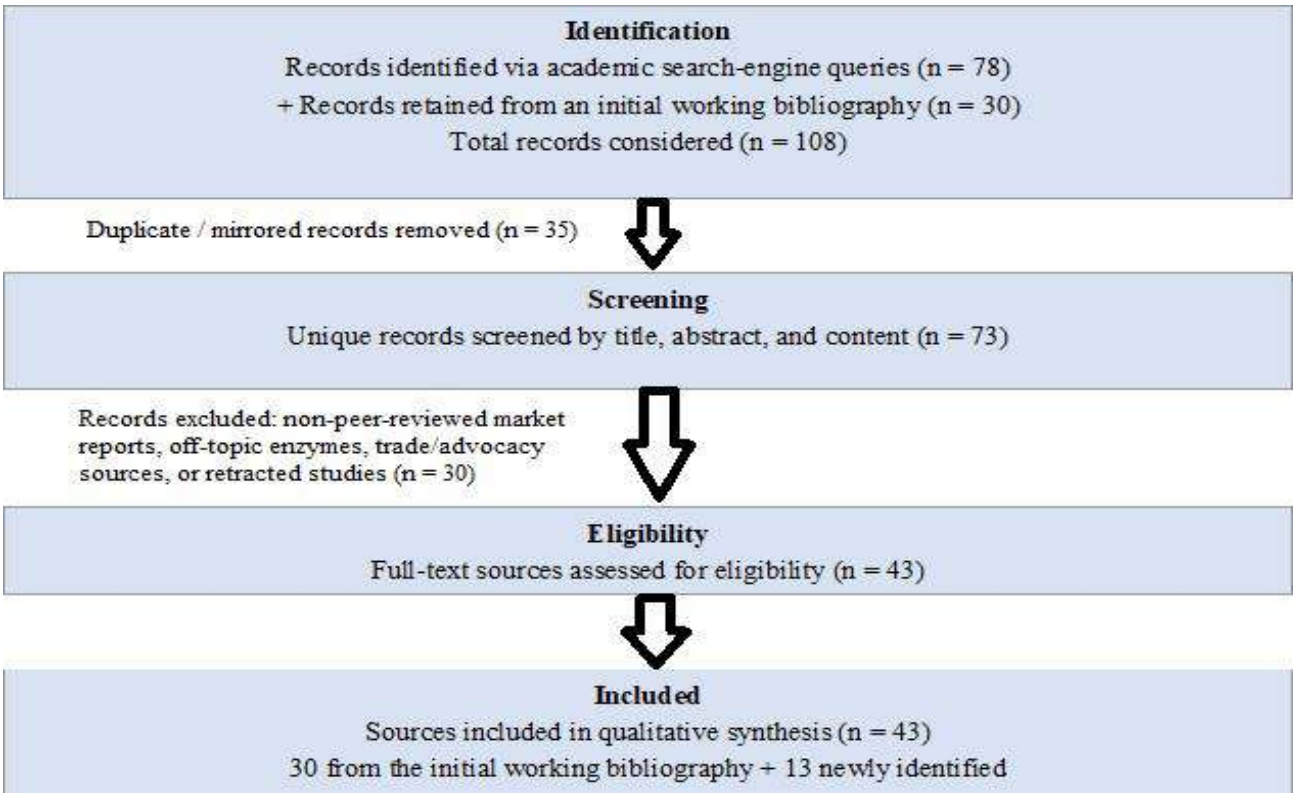
Candidate records were identified through structured queries against academic search engines and publisher/bibliographic platforms indexing PubMed/MEDLINE, Scopus, and Web of Science content, using combinations of the terms "lactase", "β-galactosidase", "lactose intolerance", "lactose-free dairy", "enzyme immobilization", "directed evolution", "protein engineering", "whey valorization", and "dairy regulation", together with citation-chaining through the reference lists of retrieved reviews (Li et al., 2023a; Zhou et al., 2024). Because this search was conducted through a general academic search tool rather than direct, exportable interrogation of each named database, the counts below should be read as an honest record of the process actually undertaken for this review rather than as deduplicated, database-specific export logs of the kind a registered PRISMA protocol would report.

Study Selection and Data Extraction

Titles, abstracts, and (where necessary) full texts were screened against the eligibility criteria above. For each included source, the following data were extracted where reported: enzyme source and operating conditions, processing or immobilization method, quantitative performance outcomes (e.g., percentage lactose reduction, retained activity after reuse), and any stated regulatory or market context. Quantitative claims reproduced in this review (e.g., activity retention percentages) are cited to the originating primary study rather than to a secondary or tertiary source.

Search and Selection Results

Figure 1 summarizes the search and selection process. A total of 78 candidate records were identified through the search strategy described above, supplemented by 30 core references drawn from an initial working bibliography compiled during background scoping of the topic. After removing duplicate or mirrored records (the same source appearing across multiple queries or host platforms), 73 unique records remained for screening. Screening against the eligibility criteria excluded 30 records, principally non-peer-reviewed commercial market reports, trade or advocacy websites, studies of enzymes unrelated to β-galactosidase, and one retracted meta-analysis that was excluded from citation despite its continued indexing. The remaining 43 sources were assessed in full text and included in the qualitative synthesis presented in this review.



Note: "Initial working bibliography" refers to the 30 core references compiled during background scoping of the topic prior to the structured database-style search described above.

Figure 1. Search and selection flow diagram

3. LACTASE: ENZYME CHARACTERISTICS, SOURCES, AND MECHANISM

Industrial lactase preparations are produced by microbial fermentation rather than extraction from mammalian tissue, since microbial sources offer higher, more controllable yields and are amenable to strain and process optimization (Taeger & Thiele, 2021; Zhou et al., 2024). The two enzyme classes used commercially differ substantially in their optimal operating conditions and applications. Yeast-derived lactase, principally from Kluyveromyces lactis, exhibits a near-neutral pH optimum (around pH 6.5–7.0) and is generally used to treat sweet milk and whey. Fungal lactase, typically from Aspergillus oryzae or Aspergillus niger, has an acidic pH optimum (around pH 3.5–4.5) and greater thermal tolerance, making it more suitable for acid whey and fermented-product applications (Costa et al., 2019; Taeger & Thiele, 2021). Bacterial β-galactosidases, including thermostable and cold-adapted variants isolated from Bacillus, Paenibacillus, and marine or psychrophilic bacteria, have attracted growing research interest because they extend the range of viable operating temperatures and can reduce the risk of microbial contamination during low-temperature processing (Ruiz-Ramírez & Jiménez-Flores, 2023; Zhou et al., 2024). Table 1 summarizes the principal enzyme sources and their characteristic operating ranges as reported across recent reviews and primary studies.

Table 1. Principal Enzyme Sources

Enzyme source	Typical optimal pH	Typical optimal temperature	Principal dairy application	Key reference(s)
Kluyveromyces lactis (yeast)	~6.5–7.0	~35–40°C	Sweet milk, low-fat milk	Krijger et al., 2012; Taeger & Thiele, 2021
Aspergillus oryzae / A. niger (fungal)	~3.5–4.5	~50–60°C	Acid whey, fermented products, yogurt	Costa et al., 2019; Miao et al., 2024; Taeger & Thiele, 2021
Bacillus spp. / Paenibacillus spp. (bacterial)	Variable, often near neutral	Mesophilic to thermophilic (up to ~65–70°C for engineered variants)	Process-integrated hydrolysis, GOS synthesis	Ruiz-Ramírez & Jiménez-Flores, 2023; Zhou et al., 2024
Psychrophilic / marine bacterial isolates	Near neutral	Cold-active (activity retained at refrigeration temperatures)	Cold-chain and low-temperature hydrolysis	Zhou et al., 2024

The choice of enzyme source is therefore not merely a matter of catalytic efficiency but of compatibility with a specific product's processing pH, temperature profile, and downstream sensory requirements, an integration challenge that is often understated in earlier reviews focused primarily on enzyme discovery.

4. CURRENT STATUS OF LACTASE APPLICATIONS IN DAIRY PRODUCTS

Lactose-Free Milk

Lactose-free milk remains the most commercially mature application, produced by adding lactase to pasteurized milk under controlled temperature and holding time to achieve near-complete hydrolysis (Dekker et al., 2019; Rizzo et al., 2020). The resulting increase in free glucose and galactose raises perceived sweetness, which manufacturers exploit to reduce added-sugar content in flavored variants (Suri et al., 2019). Ultrafiltration is increasingly combined with enzymatic treatment: concentrating lactose prior to hydrolysis increases substrate availability and reaction efficiency, yielding more consistent lactose-free end products (Kravtsov et al., 2021; Lelia & Suharoschi, 2022).

Lactose-Free Yogurt and Cheese

In yogurt manufacture, lactase can be added prior to or during fermentation; in engineered products, β-galactosidase variants with shifted pH and temperature optima have recently been used to co-optimize lactose hydrolysis and starter-culture growth, simultaneously enabling lactose-free and no-sugar-added yogurt formulations that meet national labelling thresholds for residual lactose content (Miao et al., 2024). In cheesemaking, lactase may be applied to the cheese milk before coagulation or to the curd/whey fraction post-formation; the latter approach is particularly relevant for aged cheeses in which residual lactose is low but non-zero (Capcanari et al., 2021; Suri et al., 2019).

Lactose-Free Ice Cream and Whey-Derived Products

Enzymatic treatment of the milk or cream fraction used in ice cream manufacture removes lactose without altering the creaminess or overrun characteristics that define product quality (Özdemir et al., 2018). Because whey protein powders derived from cheese manufacture retain residual lactose, lactase treatment of whey permeate, often combined with membrane separation, enables lactose-free whey protein products for sports-nutrition and clinical markets, an application whose commercial relevance is expanding alongside the broader functional-foods sector (Meehan, 2021).

 | https://doi.org/10.66529/agripat.2026.2.4.121

Infant Formula

Lactose-free and low-lactose infant formulae address secondary lactase deficiency, most commonly triggered by infectious or inflammatory enteropathy in infants and young children, rather than the genetically programmed adult-onset decline that dominates the epidemiology discussed in Section 1 (Lasekan et al., 2011; O'Connor, 2009). Formulation in this context requires particular attention to maintaining caloric density and micronutrient bioavailability once lactose, a source of both energy and prebiotic-like functionality in some contexts, is removed or substituted.

5. CHALLENGES IN THE APPLICATION OF LACTASE

Enzyme Stability: Thermal and pH Sensitivity

As a protein catalyst, lactase is intrinsically vulnerable to denaturation outside its optimal temperature and pH range (Király et al., 2021). The central process conflict is that most dairy pasteurization regimes require temperatures (e.g., ~72°C for high-temperature short-time pasteurization) well above the thermal tolerance of native yeast and fungal lactases, forcing manufacturers either to add the enzyme after thermal treatment, reintroducing microbiological risk windows, or to accept reduced catalytic efficiency (Popescu et al., 2021). Similarly, the pH of fermented products such as yogurt and cheese can drift outside the enzyme's optimal range during processing, reducing hydrolysis completeness unless carefully controlled (Popescu et al., 2021).

Production and Formulation Costs

Commercial lactase is produced by submerged or solid-state microbial fermentation followed by purification, both of which are capital- and energy-intensive at industrial scale (Mahalakshmi et al., 2013; Taeger & Thiele, 2021). Formulation for storage stability often requires stabilizing excipients and protective packaging, adding further cost (Mahalakshmi et al., 2013). Techno-economic analyses of lactose-reduced diets indicate that lactose-free substitutes carry a measurable price premium over conventional dairy, which can be a barrier to adoption in lower-income settings even where the underlying prevalence of lactose intolerance is highest (Taeger & Thiele, 2021).

Consumer Acceptance and Sensory Perception

Consumer research indicates that acceptance of lactose-free dairy is shaped less by objective sensory differences, which are generally minor, than by perceptions regarding the naturalness of enzymatic processing and unfamiliarity with the concept of enzyme-treated food (Rizzo et al., 2020). Continuous improvement of enzyme formulations to minimize any residual sweetness or texture deviation, combined with transparent labelling, remains important for market expansion, particularly outside the North American and European markets where lactose-free products have historically been concentrated (Kárnyáczki & Csanádi, 2017; Rizzo et al., 2020).

Regulatory Heterogeneity

Perhaps the least appreciated barrier to international scale-up is the lack of harmonized thresholds for “lactose-free” and “low-lactose” labelling. As summarized in Table 2, permissible residual lactose concentrations vary by an order of magnitude or more between jurisdictions, complicating compliance for manufacturers operating in multiple markets and creating potential for consumer confusion when products are traded internationally (Li et al., 2023a; Ronchetti et al., 2024).

Table 2. Illustrative Residual Lactose Thresholds for “Lactose-Free” and “Low-Lactose” Labelling

Jurisdiction / authority	Threshold	Label category
European Union (EFSA guidance)	< 1,000 mg/L lactose	“Lactose-free”
China	< 500 mg/100 mL (≈ 5,000 mg/L)	“Lactose-free”
India – Food Safety and Standards Authority of India (FSSAI), under the Food Safety and Standards Regulations	< 10,000 mg/L	“Low-lactose”
India – Food Safety and Standards Authority of India (FSSAI), under the Food Safety and Standards Regulations	< 100 mg/L	“Lactose-free”
Italy (Ministry of Health)	0.1% w/w	“Lactose-free”

Source: thresholds compiled and reported by Li et al. (2023a). The two India entries reflect different threshold tiers (“low-lactose” versus “lactose-free”) set within a single national regulatory framework administered by FSSAI, not two separate regulatory bodies, as earlier drafts of this table implied. Values are illustrative of the degree of international variation rather than an exhaustive regulatory inventory; because they are drawn from a secondary compilation and could not be independently cross-checked against primary Indian gazette notifications during this revision, manufacturers should verify current requirements directly with the relevant national authority before regulatory or labelling use, as thresholds are periodically revised.

 | https://doi.org/10.66529/agripat.2026.2.4.121

Safety evaluation of lactase preparations themselves, covering allergenicity, production-strain characterization, and absence of mycotoxin or endotoxin contamination, is a further prerequisite for market approval. In the European Union, this evaluation is carried out formally by the European Food Safety Authority under the food enzyme Regulation (EC) No. 1332/2008, which requires a dossier addressing production-strain identity, manufacturing process, composition, and allergenicity before a food enzyme is placed on the Union list (EFSA CEP Panel et al., 2023). In the United States, food enzymes such as lactase are typically brought to market through the Generally Recognized as Safe (GRAS) notification pathway administered by the U.S. Food and Drug Administration. These parallel but non-identical evidentiary standards contribute to the same cross-jurisdictional compliance burden already noted above (Khelef et al., 2022).

6. RECENT ADVANCES IN ENZYME ENGINEERING AND IMMOBILIZATION

Protein Engineering and Directed Evolution

Rational protein engineering and directed evolution have been used to address the intrinsic limitations of wild-type β-galactosidases, including modest thermostability, narrow pH tolerance, and product (galactose) inhibition, which slows the later stages of hydrolysis reactions (Liu et al., 2025). Site-saturation mutagenesis of Aspergillus oryzae β-galactosidase (AoBgal35A), for example, produced a T955R variant with a lactose hydrolysis rate of 90.7% compared with 78.5% for the wild-type enzyme, alongside a shift in optimal pH from 4.5 to 5.5 and a reduction in optimal temperature from 60°C to 50°C, changes that better align the enzyme with fermented-dairy processing conditions and were used successfully to manufacture lactose-free, no-sugar-added yogurt (Miao et al., 2024). More broadly, disulfide-bond engineering and domain-fusion strategies applied to Kluyveromyces lactis and other industrially relevant β-galactosidases have improved thermal half-life at process-relevant temperatures, though most such gains remain confined to laboratory- and pilot-scale demonstration rather than validated industrial deployment (Liu et al., 2025).

Enzyme Immobilization

Immobilization, attaching lactase to a solid support via adsorption, covalent binding, cross-linking, entrapment, or encapsulation, improves operational reusability, simplifies downstream product separation, and can enhance resistance to thermal and chemical denaturation (Kárnyáczki & Csanádi, 2017; Zhou et al., 2024). An early demonstration of this principle used lactase immobilized in a glass-microsphere packed-bed reactor to achieve continuous-flow lactose-free milk production, establishing reactor-based proof-of-concept ahead of the nanostructured supports discussed below (Ko et al., 2018). Several more recent studies illustrate the range of supports now under investigation and the magnitude of achievable improvement. Covalent immobilization of β-galactosidase onto alginate/tea-waste composite beads, an approach that simultaneously valorizes an agro-industrial by-product, improved catalytic stability while maintaining favorable effects on treated dairy product quality (Hassan et al., 2024). Immobilization on zeolitic imidazolate framework (ZIF-L) metal–organic-framework supports retained 86% of initial enzymatic activity after four consecutive reuse cycles, demonstrating the potential of nanostructured inorganic carriers for continuous-flow applications (Al-Meetani et al., 2025). Core-shell electrospun polymer nanofibers, using a protective outer shell around an aqueous enzyme-containing core, achieved a 50% reduction in lactose within the first hour of treatment in whole milk, compared with 20% for the free enzyme under identical conditions, and a 56% lactose reduction when combined with alginate co-encapsulation in low-fat milk, illustrating that support architecture can materially accelerate early-stage hydrolysis kinetics even where longer-term endpoints converge (Peta et al., 2025). The shell material used in this nanofiber system is non-biodegradable and comes into direct contact with the treated milk; food-contact-material safety, including potential migration of shell polymer or degradation products into the dairy matrix, has not yet been systematically evaluated for this architecture, and formal assessment under applicable food-contact regulations (e.g., EU Regulation (EC) No. 1935/2004 or the equivalent national framework) would be a prerequisite for industrial adoption (Peta et al., 2025). Complementing these empirical support-development studies, kinetic modeling comparing immobilized Aspergillus niger lactase with the free enzyme has been used to identify which reaction models (e.g., Michaelis–Menten formulations incorporating competitive product inhibition) best describe hydrolysis behavior once the enzyme is immobilized, information that is directly useful for reactor design (Mirsalami & Alihosseini, 2021). Table 3 summarizes the principal immobilization strategies, their operating principle, and their reported trade-offs.

Collectively, these results indicate genuine, measurable progress since earlier reviews that described immobilization largely in prospective terms; however, comparative data across support types under identical, industrially representative conditions remain scarce, which limits the ability to recommend a single “best” approach across product categories (Al-Meetani et al., 2025; Peta et al., 2025; Zhou et al., 2024).

 | https://doi.org/10.66529/agripat.2026.2.4.121

Table 3. Principal Immobilization Strategies

Technique	Operating principle	Reported advantages	Reported limitations
Adsorption	Non-covalent binding to support surface	Simple, low-cost, mild conditions	Enzyme leaching under process shear/ionic strength changes
Covalent binding	Chemical bond between enzyme and functionalized support	High operational stability; low leaching	Risk of active-site distortion; higher preparation cost
Entrapment / encapsulation	Enzyme physically enclosed within a polymer matrix (e.g., alginate, electrospun nanofibers)	Protects enzyme from denaturants; enables controlled release	Diffusional limitations can reduce apparent activity
Cross-linking	Enzyme molecules linked to one another (e.g., CLEAs) without a carrier	No carrier cost; high volumetric activity	Mechanical fragility; variable reproducibility
Metal–organic framework (MOF) support	Enzyme hosted within or on a crystalline porous framework (e.g., ZIF-L)	High surface area; strong reuse stability (e.g., 86% activity retained after 4 cycles)	Emerging technology; scale-up and food-contact safety data still limited

7. COST-EFFECTIVE AND SUSTAINABLE PRODUCTION

Reducing the cost of lactase production is essential for broadening access to lactose-free dairy, particularly in the high-prevalence, often lower-income regions where the burden of lactose intolerance is greatest (Taeger & Thiele, 2021). Two complementary strategies are prominent in recent literature. First, genetic and metabolic engineering of production strains including Kluyveromyces lactis and filamentous fungi continue to be used to raise volumetric enzyme yield and reduce fermentation time (Krijger et al., 2012). Second, and increasingly emphasized, is the use of dairy-processing by-products, particularly cheese whey, as a low-cost fermentation substrate for lactase-producing microorganisms, closing a material loop within the dairy value chain (Greta et al., 2024; Lappa et al., 2021). Whey remains one of the food industry's largest by-product streams by volume, and its use both as a fermentation feedstock for lactase production and as the substrate ultimately treated by that lactase exemplifies a circular-economy approach that simultaneously addresses waste-management and production-cost objectives (Lappa et al., 2021). Complementary integrated schemes have also been proposed in which lactase-mediated hydrolysis of dairy waste streams is coupled directly with downstream conversion into functional food ingredients, extending this circular-economy rationale beyond enzyme production alone (Palai & Dubey, 2022). Solid-state fermentation using agricultural by-products as a lactase-production substrate has similarly been explored as a lower-cost alternative to submerged fermentation; optimized solid-state fermentation conditions for high-yield β-galactosidase-producing lactic acid bacteria have been reported to substantially raise volumetric enzyme yield for downstream use in low-lactose dairy manufacture, although reported yields and scalability vary considerably between studies and strains (Zhang, 2025).

Green biocatalysis principles minimizing solvent use, favoring energy-efficient membrane-based purification, and recovering process heat are increasingly cited as sustainability benchmarks for enzyme manufacturing, though few published studies report full life-cycle assessment data for lactase production specifically, representing a notable evidence gap addressed further in Section 9 (Ottone et al., 2021).

8. INTEGRATION WITH COMPLEMENTARY PROCESSING TECHNOLOGIES

Combining lactase treatment with other unit operations can improve overall process efficiency and product quality. Ultrafiltration concentrates lactose ahead of enzymatic treatment, increasing substrate availability and hydrolysis consistency (Kravtsov et al., 2021). Microencapsulation protects lactase from adverse environmental conditions during storage and processing while enabling gradual, controlled enzyme release within the product matrix (Kravtsov et al., 2021). High-pressure processing, a non-thermal preservation technology, has been investigated for its potential to modulate enzyme conformation and activity while simultaneously achieving microbial inactivation, offering a possible route to extended shelf-life lactose-free products without conventional thermal pasteurization trade-offs, although the enzyme-activation mechanism under high pressure is not yet fully characterized and warrants further mechanistic study (Liepa et al., 2016). At the fermentation-control level, advanced process-control strategies including model-reference adaptive control of batch fermentation bioreactors have also been proposed to keep temperature and pH within the narrow windows that lactase and starter cultures both require, illustrating that integration challenges extend beyond unit-operation sequencing to real-time process control (Ritonja et al., 2020).

 | https://doi.org/10.66529/agripat.2026.2.4.121

9. RESEARCH GAPS AND CRITICAL PERSPECTIVE

While the preceding sections document substantial technical progress, this review identifies several gaps that constrain translation of laboratory advances into industrial practice and that should inform future research priorities.

- Techno-economic evidence: Few published studies report direct, standardized techno-economic comparisons between immobilization strategies, engineered-enzyme variants, and conventional free-enzyme processes under equivalent industrial conditions, making it difficult for manufacturers to prioritize investment.
- Lifecycle and environmental data: Claims regarding the sustainability benefits of green biocatalysis and whey valorization are frequently qualitative; quantitative life-cycle assessment data specific to lactase production and application remain sparse.
- Geographic and demographic representativeness: Consumer-acceptance research is concentrated in North American and European markets, despite the highest prevalence of lactose intolerance occurring in Asian, African, and Indigenous American populations that are comparatively under-studied and under-served by current product ranges.
- Personalized nutrition: Nutrigenomic tailoring of lactase supplementation to individual lactase-persistence genotype is conceptually promising but remains largely aspirational in the peer-reviewed literature; robust clinical validation of personalized dosing algorithms has not yet been demonstrated at scale.
- Regulatory harmonization research: There is a shortage of comparative regulatory-science literature analyzing the practical consequences of threshold heterogeneity (Table 2) for international trade, labelling accuracy, and consumer trust.

Addressing these gaps will require interdisciplinary collaboration extending beyond enzymology and food process engineering to include health economics, life-cycle analysis, regulatory science, and cross-cultural consumer research.

10. CONCLUSION

Lactase-mediated hydrolysis has transformed the accessibility of dairy products for a substantial fraction of the global population affected by lactose intolerance, enabling lactose-free milk, yogurt, cheese, ice cream, whey protein, and infant formula that retain much of the nutritional profile of conventional dairy (Dominici et al., 2022; Li et al., 2023a). Meaningful progress has been achieved in enzyme engineering through directed evolution and rational mutagenesis that shift pH and temperature optima toward process-relevant conditions, and in immobilization technology, where metal–organic-framework and electrospun-polymer supports have demonstrated quantifiable gains in operational stability and reuse (Al-Meetani et al., 2025; Liu et al., 2025; Miao et al., 2024; Peta et al., 2025). Nonetheless, cost, thermal and pH sensitivity, consumer perception, and international regulatory heterogeneity continue to constrain broader and more equitable deployment, particularly in the high-prevalence regions where need is greatest (Li et al., 2023a; Taeger & Thiele, 2021). Future progress will depend less on isolated enzyme-discovery breakthroughs than on integrating protein engineering, sustainable and whey-valorizing production strategies, complementary processing technologies, and harmonized regulatory and consumer-research evidence into a coherent innovation pathway for the dairy industry.

References

Al-Meetani, B., Almadhaani, R., Salim, S.A., Hassan, A., Javed, F., & Al-Zuhair, S. (2025). Enhanced stability, reusability, and lactose hydrolysis of β-galactosidase immobilized in ZIF-L frameworks. Journal of Chemical Technology & Biotechnology. Advance online publication. https://doi.org/10.1002/jctb.70035

Capcanari, T., Chirsanova, A., Covaliov, E., & Siminiuc, R. (2021). Development of lactose free yogurt technology for personalized nutrition. Food and Nutrition Sciences, 12(11), 1116–1135. https://doi.org/10.4236/fns.2021.1211082

Costa, A., Lopez-Villalobos, N., Sneddon, N.W., Shalloo, L., Franzoi, M., De Marchi, M., & Penasa, M. (2019). Invited review: Milk lactose—Current status and future challenges in dairy cattle. Journal of Dairy Science, 102(7), 5883–5898. https://doi.org/10.3168/jds.2018-15955

Dekker, P.J., Koenders, D., & Bruins, M.J. (2019). Lactose-free dairy products: Market developments, production, nutrition and health benefits. Nutrients, 11(3), 551. https://doi.org/10.3390/nu11030551

Dominici, S., Marescotti, F., Sanmartin, C., Macaluso, M., Taglieri, I., Venturi, F., Zinnai, A., & Facioni, M.S. (2022). Lactose: Characteristics, food and drug-related applications, and its possible substitutions in meeting the needs of people with lactose intolerance. Foods, 11(10), 1486. https://doi.org/10.3390/foods11101486

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J.M., Bolognesi, C., Cocconcelli, P.S., Crebelli, R., Gott, D.M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Herman, L., Aguilera, J., Andryszkiewicz, M., Liu, Y., & Chesson, A. (2023).

Safety evaluation of the food enzyme β-galactosidase from the non-genetically modified Kluyveromyces lactis strain GD-YNL. EFSA Journal, 21(1), e07750. https://doi.org/10.2903/j.efsa.2023.7750

Greta, B., Alex, P., Diletta, A., Samuele, S., Marcella, D., Antonino, N., Marina, L., Luca, B., Stefania, B., & Marco, M. (2024). Sustainable production of a biotechnologically relevant β-galactosidase in Escherichia coli cells using crude glycerol and cheese whey permeate. Bioresource Technology, 406, Article 131063. https://doi.org/10.1016/j.biortech.2024.131063

Hassan, M.E., Ibrahim, G.E., & Abdella, M.A.A. (2024). Enhancement of β-galactosidase catalytic activity and stability through covalent immobilization onto alginate/tea waste beads and evaluating its impact on the quality of some dairy products. International Journal of Biological Macromolecules, 278, Article 134810. https://doi.org/10.1016/j.ijbiomac.2024.134810

Kárnyáczki, Z., & Csanádi, J. (2017). Texture profile properties, sensory evaluation, and susceptibility to syneresis of yoghurt prepared from lactose-free milk. Acta Alimentaria, 46(4), 403–410. https://doi.org/10.1556/066.2016.0018

Khelef, M., Golubtsova, Y.V., & Ivanova, S.A. (2022). Lactose-free dairy products: Production prospects. New Technologies, 18(3), 94–105. https://doi.org/10.47370/2072-0920-2022-18-3-94-105

Király, M., Kiss, B.D., Horváth, P., Drahos, L., Mirzahosseini, A., Pálfy, G., Antal, I., & Ludányi, K. (2021). Investigating thermal stability based on the structural changes of lactase enzyme by several orthogonal methods. Biotechnology Reports, 30, Article e00637. https://doi.org/10.1016/j.btre.2021.e00637

Ko, C.Y., Liu, J.M., Chen, K.I., Hsieh, C.W., Chu, Y.L., & Cheng, K.C. (2018). Lactose-free milk preparation by immobilized lactase in glass microsphere bed reactor. Food Biophysics, 13, 353–361. https://doi.org/10.1007/s11483-018-9541-8

Kravtsov, V.A., Kulikova, I.K., Anisimov, G.S., Evdokimov, I.A., & Khramtsov, A.G. (2021). Variety of dairy ultrafiltration permeates and their purification in lactose production. IOP Conference Series: Earth and Environmental Science, 677, Article 032001. https://doi.org/10.1088/1755-1315/677/3/032001

Krijger, J.J., Baumann, J., Wagner, M., Schulze, K., Reinsch, C., Klose, T., Onuma, O.F., Simon, C., Behrens, S.E., & Breunig, K.D. (2012). A novel, lactase-based selection and strain improvement strategy for recombinant protein expression in Kluyveromyces lactis. Microbial Cell Factories, 11, Article 112. https://doi.org/10.1186/1475-2859-11-112

Lappa, I.K., Kachrimanidou, V., Papadaki, A., Stamatiou, A., Ladakis, D., Eriotou, E., & Kopsahelis, N. (2021). A comprehensive bioprocessing approach to foster cheese whey valorization: On-site β-galactosidase secretion for lactose hydrolysis and sequential bacterial cellulose production. Fermentation, 7(3), 184. https://doi.org/10.3390/fermentation7030184

Lasekan, J.B., Jacobs, J., Reisinger, K.S., Montalto, M.B., Frantz, M.P., & Blatter, M.M. (2011). Lactose-free milk protein-based infant formula: Impact on growth and gastrointestinal tolerance in infants. Clinical Pediatrics, 50(4), 330–337. https://doi.org/10.1177/0009922810390511

Lelia, P.O., & Suharoschi, R. (2022). Emerging food processing technologies: Probiotics and prebiotics. In Nutraceutical and functional food components (pp. 509–536). Elsevier. https://doi.org/10.1016/B978-0-323-85052-0.00008-8

Li, A., Zheng, J., Han, X., Yang, S., Cheng, S., Zhao, J., Zhou, W., & Lu, Y. (2023a). Advances in low-lactose/lactose-free dairy products and their production. Foods, 12(13), 2553. https://doi.org/10.3390/foods12132553

Li, A., Zheng, J., Han, X., Jiang, Z., Yang, B., Yang, S., Zhou, W., Li, C., & Sun, M. (2023b). Health implication of lactose intolerance and updates on its dietary management. International Dairy Journal, 140, Article 105608. https://doi.org/10.1016/j.idairyj.2023.105608

Liepa, M., Zagorska, J., & Galoburda, R. (2016). High-pressure processing as novel technology in dairy industry: A review. Research for Rural Development, 1(1), 76–83.

Liu, P., Chen, Y., Ma, C., Ouyang, J., & Zheng, Z. (2025). β-Galactosidase: A traditional enzyme given multiple roles through protein engineering. Critical Reviews in Food Science and Nutrition, 65(7), 1306–1325. https://doi.org/10.1080/10408398.2023.2292282

Mahalakshmi, S., Kiran, K.K., Hameeda, B., & Gopal, R. (2013). Fermentative production of lactase from Lactobacillus amylophilus GV6. Journal of Scientific & Industrial Research, 72, 548–552.

Malik, T.F., & Panuganti, K.K. (2024). Lactose intolerance. Institute of Dairy Nutrition and Health. https://www.frieslandcampinainstitute.com/uploads/sites/19/2024/09/FC-Institute-Publication-on-Lactose-intolerance.pdf

Meehan, S. (2021). Examination of the lactose content of 'Lactose Free' whey protein based sports nutrition products [Bachelor's thesis, University of Veterinary Medicine Budapest]. Hungarian Veterinary and Agricultural Digital Archive (HuVetA). http://huveta.hu/handle/10832/3144

Miao, M., Li, S., Yang, S., Yan, Q., Xiang, Z., & Jiang, Z. (2024). Engineering the β-galactosidase from Aspergillus oryzae for making lactose-free and no-sugar-added yogurt. Journal of Dairy Science, 107(9), 6602–6613. https://doi.org/10.3168/jds.2023-24310

Mirsalami, S.M., & Alihosseini, A. (2021). Selection of the most effective kinetic model of lactase hydrolysis by immobilized Aspergillus niger and free β-galactosidase. Journal of Saudi Chemical Society, 25(12), Article 101395. https://doi.org/10.1016/j.jscs.2021.101395

Novalin, S., Neuhaus, W., & Kulbe, K.D. (2005). A new innovative process to produce lactose-reduced skim milk. Journal of Biotechnology, 119(2), 212–218. https://doi.org/10.1016/j.jbiotec.2005.03.018

O'Connor, N.R. (2009). Infant formula. American Family Physician, 79(7), 565–570.

Ottone, C., Romero, O., Urrutia, P., Bernal, C., Illanes, A., & Wilson, L. (2021). Enzyme biocatalysis and sustainability. In Nanostructured catalysts for environmental applications (pp. 383–413). Springer. https://doi.org/10.1007/978-3-030-58934-9_14

Özdemir, C., Arslaner, A., Özdemir, S., & Uğurlu, G. (2018). Ice-cream production from lactose-free UHT milk. Journal of Food Science and Engineering, 8, 210–214. https://doi.org/10.17265/2159-5828/2018.05.003

Palai, T., & Dubey, K.K. (2022). Valorization of dairy industry waste into functional foods using lactase. In P. Verma (Ed.), Thermochemical and catalytic conversion technologies for future biorefineries (Vol. 2, pp. 161–183). Springer. https://doi.org/10.1007/978-981-19-4316-4_7

Peta, G., Avrahami, R., Zussman, E., Rokem, J.S., & Greenblatt, C. (2025). Immobilization of enzymes for synergy in polymers to produce lactose free milk. Scientific Reports, 15(1), 17618. https://doi.org/10.1038/s41598-025-00172-6

Popescu, L., Bulgaru, V., & Siminiuc, R. (2021). Effect of temperature, pH and amount of enzyme used in the lactose hydrolysis of milk. Food and Nutrition Sciences, 12(12), 1243–1254. https://doi.org/10.4236/fns.2021.1212091

Ritonja, J., Goršek, A., & Pečar, D. (2020). Model reference adaptive control for milk fermentation in batch bioreactors. Applied Sciences, 10(24), 9118. https://doi.org/10.3390/app10249118

Rizzo, P., Harwood, W., & Drake, M. (2020). Consumer desires and perceptions of lactose-free milk. Journal of Dairy Science, 103(8), 6950–6966. https://doi.org/10.3168/jds.2019-17940

Ronchetti, F., Springer, L., & Purnhagen, K. (2024). The regulatory landscape in the EU for dairy products derived from precision fermentation. SpringerBriefs in Law. https://www.springerprofessional.de/en/the-regulatory-landscape-in-the-eu-for-dairy-products-derived-fr/26683418

Ruiz-Ramírez, S., & Jiménez-Flores, R. (2023). Invited review: Properties of β-galactosidases derived from Lactobacillaceae species and their capacity for galacto-oligosaccharide production. Journal of Dairy Science, 106(12), 8193–8206. https://doi.org/10.3168/jds.2023-23392

Sharp, E., D'Cunha, N.M., Ranadheera, C.S., Vasiljevic, T., Panagiotakos, D.B., & Naumovski, N. (2021). Effects of lactose-free and low-lactose dairy on symptoms of gastrointestinal health: A systematic review. International Dairy Journal, 114, Article 104936. https://doi.org/10.1016/j.idairyj.2020.104936

Suri, S., Kumar, V., Prasad, R., Tanwar, B., Goyal, A., Kaur, S., Gat, Y., Kumar, A., Kaur, J., & Singh, D. (2019). Considerations for development of lactose-free food. Journal of Nutrition & Intermediary Metabolism, 15, 27–34. https://doi.org/10.1016/j.jnim.2018.11.003

Swagerty, D.L., Walling, A.D., & Klein, R.M. (2002). Lactose intolerance. American Family Physician, 65(9), 1845–1851.

Taeger, M., & Thiele, S. (2021). Additional costs of lactose-reduced diets: Lactose-free dairy product substitutes are a cost-effective alternative for people with lactose intolerance. Public Health Nutrition, 24(13), 4043–4053. https://doi.org/10.1017/S1368980021002779

Zhang, Z. (2025). Optimization of solid-state fermentation conditions for high β-galactosidase-producing lactic acid bacteria and its application in low-lactose dairy products. Frontiers in Bioengineering and Biotechnology, 13, Article 1708601. https://doi.org/10.3389/fbioe.2025.1708601

Zhou, Y., Liu, Y., Gao, F., Xia, Z., Zhang, Z., Addai, F.P., Zhu, Y., Chen, J., Lin, F., & Chen, D. (2024). β-Galactosidase: Insights into source variability, genetic engineering, immobilisation and diverse applications in food, industry and medicine. International Journal of Dairy Technology, 77(3), 651–679. https://doi.org/10.1111/1471-0307.13098

Declarations

Funding: The authors declare no specific funding for this work.

Conflict of Interest: The authors declare no conflict of interest.

Author Contributions: *Raza, A.* – Conceptualization, literature review and synthesis, writing (original draft), and critical revision of the manuscript. *Xiaodong, L.* – Literature analysis, synthesis of evidence, and writing (review and editing). *Amjad, A.* – Literature review, analysis, and writing (review and editing). *Haq, F.U.* – Critical analysis, interpretation of the literature, and writing (review and editing). *Wang, Z.* – Supervision, conceptualization, critical revision, and final approval of the manuscript.

Use of AI: AI-assisted tools (LLMs) were used to improve the language and grammar of this paper. The authors reviewed and approved all content and assume full responsibility for its accuracy.

