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Review

Modulation of Enzymatic Activity by Moderate Electric Fields: Perspectives for Prebiotic Epilactose Production via Cellobiose-2-Epimerase

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Abstract

Modulating enzymatic activity through physical strategies is increasingly recognized as a powerful approach to optimizing biocatalytic processes in food and biotechnology applications. Cellobiose 2-epimerase (C2E), a key enzyme for synthesizing epilactose, a non-digestible disaccharide with established prebiotic effects, is gaining relevance in functional foods. Emerging strategies, such as the application of moderate electric fields (MEFs), have attracted attention due to their non-thermal, non-invasive nature and their capacity to influence the structural and functional properties of proteins. This review assesses the potential of MEFs to modulate C2E activity and provides an overview of the physicochemical principles governing MEF–protein interactions and summarizes findings from various enzymatic systems, highlighting changes in activity, stability, and substrate affinity under electric field conditions. Particular attention is given to the mechanistic plausibility and processing implications of applying MEFs to C2E-catalyzed reactions. The integration of biochemical, structural, and engineering perspectives suggests that MEF-assisted modulation could overcome current bottlenecks in epilactose production. This approach may enable the sustainable valorization of lactose-rich byproducts and support the development of non-thermal, clean-label technologies for producing functional ingredients.

Keywords: cellobiose 2-epimerase; epilactose; lactose epimerization; prebiotic sugars; enzymatic modulation

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1. Introduction

Cellobiose-2-epimerase (C2E) has attracted interest in the field of biotechnology due to its ability to catalyze the reversible epimerization of lactose into epilactose, a rare disaccharide with promising prebiotic properties. These include resistance to upper gastrointestinal digestion and selective fermentation that stimulates beneficial gut microbiota [1]. This enzyme, originally identified in thermophilic microorganisms, *Ruminococcus albus*, *Caldicellulosiruptor saccharolyticus*, and *Thermoanaerobacterium thermosaccharolyticum* [2,3], has been studied for its potential in industrial processes targeting the production of functional sugars, particularly in response to the growing demand for foods that support gastrointestinal and immune health.

Epilactose stands out for its potential to selectively promote the growth of bifidobacteria and several butyrate-producing bacteria [4]. In in vitro fermentation trials, it induced up to 70-fold higher butyrate production compared to lactulose, suggesting fewer laxative effects and greater functional value [4]. This makes epilactose an attractive ingredient for functional food formulations. Its fermentation profile is characterized by increased butyrate production; a metabolite associated with gut and immune health [1]. However, other short-chain fatty acids (SCFAs), such as acetate and propionate, have also been identified as relevant metabolites generated during the fermentation of epilactose by gut microbiota [1,4]. Studies have demonstrated that epilactose positively modulates the human gut microbiota by increasing *Actinobacteria* and *Firmicutes* and reducing *Proteobacteria*, which generally includes intestinal pathogens [4]. The industrial potential of prebiotics such as galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) is underscored by the rapid market expansion of this sector. The global prebiotics market was valued at USD 8.5 billion in 2023 and is projected to reach USD 21.8 billion by 2030, with a compound annual growth rate (CAGR) of 14.3% [5]. Similarly, the global prebiotic ingredients market, which encompasses GOS, FOS, and emerging functional sugars such as epilactose, was valued at USD 9.0 billion in 2024 and is expected to reach USD 28.6 billion by 2034 [6]. These figures highlight the significant commercial opportunities for innovative prebiotic compounds. However, the efficient industrial-scale production of epilactose still faces significant challenges, such as low conversion rates, enzyme inactivation under processing conditions, and the need for costly purification steps [7].

Various strategies have been explored to optimize the catalytic performance and stability of industrial enzymes, including enzyme immobilization [8], reaction medium modification [9], protein engineering [10], and the use of cofactors [11]. Nevertheless, these approaches often involve chemical modifications, cost-intensive steps, or long development times. In recent years, an emerging approach, moderate electric fields (MEFs), has shown promising potential to modulate enzymatic activity [12]. MEFs are based on the passage of an alternating current through the sample at defined electrical frequencies (i.e., from Hz to kHz). Depending on the electrical protocol applied, these treatments may either generate internal heat (known as the ohmic heating effect) or induce physical and electrochemical effects due to the presence of electric fields. In some cases, a combination of thermal, electrical, and chemical effects can occur (Brochier et al., 2016) [13]. In general, the term MEFs is used to describe treatments in which thermal effects are minimized or avoided [14]. MEFs promote precise conformational changes in proteins, which may enhance catalytic activity, thermal stability, or reaction selectivity [15].

Although no direct evidence is yet available for C2E, certain structural features observed in epimerases from the same family—such as a well-defined dependence on divalent metal cofactors, the presence of flexible loops adjacent to the active site, and clusters of charged residues near the catalytic center—suggest a potential responsiveness to MEFs [3,16]. These traits are known to influence protein conformational flexibility and electrostatic interactions, which could, in principle, enhance susceptibility to MEF-induced modulation of spatial orientation, catalytic dynamics, and reaction selectivity [17]. This gap is particularly relevant for enzymes like C2E, whose industrial performance generally remains limited by suboptimal catalytic efficiency. Despite the increasing number of studies investigating the application of MEFs in enzyme modulation, there is currently no systematic review focused specifically on epimerases such as C2E or on their use in intensifying epilactose production. Given the biotechnological relevance of this application, a critical and integrated assessment of the mechanisms through which MEFs act on enzymes and how this technology could be incorporated into more efficient and sustainable biocatalytic processes is warranted.

In this context, the present review aims to summarize the state of the art on C2E and its application in epilactose production, to discuss the principles of MEFs and their effects on enzymatic systems, and to explore future perspectives for the use of MEFs as a tool to intensify biocatalytic processes for the synthesis of functional sugars. Notably, no studies to date have evaluated the direct effects of MEFs on C2E activity. This review aims to address this gap by examining MEF-induced effects on other enzymes, thereby providing indirect insights into potential mechanisms of action on C2E. Addressing this gap may unlock new possibilities for bioprocess intensification and the rational design of functional ingredients.

2. Epilactose: Properties and Production Methods

2.1. Structure, Physiological Effects and Prebiotic Action

Epilactose is a C2 epimer of lactose composed of a galactose unit linked to a D-mannose unit via a β-(1→4) bond. The inversion at C-2 alters enzyme recognition and confers distinctive physicochemical traits, including high water solubility and good thermal stability under moderate heat, ensuring compatibility with industrial processes such as pasteurization [2,3,18,19].

This disaccharide is resistant to hydrolysis by digestive enzymes, escaping metabolism in the upper gastrointestinal tract and reaching the colon intact, where it is selectively fermented by commensal anaerobes [1]. Fermentation produces short-chain fatty acids (SCFAs)—notably butyrate and, in some cases, propionate—while maintaining relatively low gas output in in vitro models [1,4,20,21]. These metabolites support gut barrier integrity, immune modulation, and anti-inflammatory effects, as detailed in later sections. Together with its good tolerability and safety profile supported by in vitro and pre-clinical data, these properties reinforce epilactose potential as a functional ingredient for applications in food and nutrition [21].

Comparative studies demonstrate that epilactose exhibits a distinct fermentation profile. Cardoso et al. (2024) [4] and Zhang et al. (2023) [1] reported that, compared with lactulose and raffinose, epilactose produced 2.4–3.2-fold higher total SCFA and lactate levels after 48 h fermentation, with a marked increase in butyrate and propionate, while the other prebiotics favored acetate and lactate. In omnivorous donors, butyrate levels were 70- and 63-fold higher than with lactulose and raffinose, respectively; in vegan donors, these increases reached 29- and 89-fold. Both studies also showed that epilactose selectively stimulated butyrate-producing bacteria (e.g., *Faecalibacterium*, *Anaerostipes*, *Intestinimonas*) and beneficial genera such as *Bifidobacterium* and *Blautia*, whereas lactulose had a more limited effect, preferentially increasing *Bifidobacterium* and *Lactobacillus* (Figure 1). In this context, MEF treatments, by enhancing C2E catalytic efficiency or stability during processing, could increase the yield and purity of epilactose in the final product. This, in turn, might influence its fermentation kinetics in the colon, potentially shifting the balance of SCFA production toward butyrate and propionate or modifying gas output. Such modulation could allow tailoring epilactose prebiotic profile to specific health or functional food applications.

Animal studies reinforce these findings. Watanabe et al. (2008) [22] reported classical prebiotic effects in rats fed 4.5% epilactose, including increased cecal weight, reduced pH, proliferation of lactobacilli and bifidobacteria, and suppression of harmful bacteria such as *Clostridia* and *Bacteroidetes*. Epilactose also inhibited the conversion of primary to secondary bile acids, a protective effect given their association with inflammation and colon carcinogenesis. Additional studies by Nishimukai et al. (2008) [23] and Suzuki et al. (2010a, 2010b) [24,25] showed enhanced calcium and iron absorption via phosphorylation of myosin light chains at epithelial junctions, reduced non-HDL cholesterol, prevention of osteopenia and anemia, and maintenance of bone mineral density.

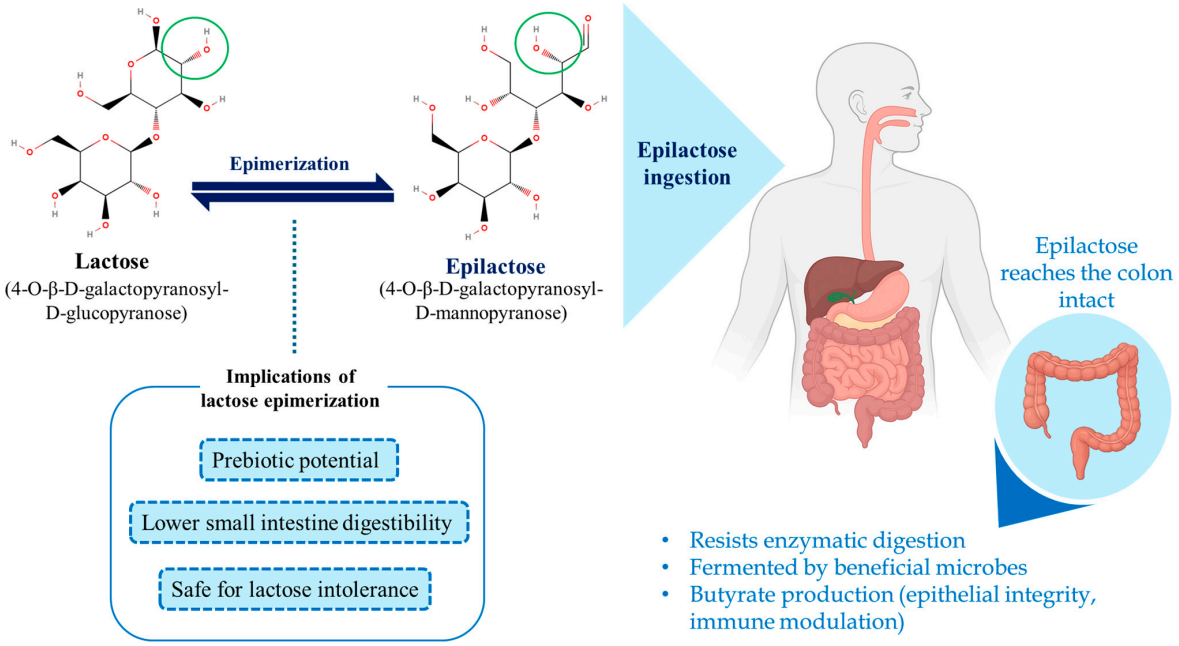


Figure 1. Structural comparison between lactose and epilactose and schematic representation of epilactose fermentation in the colon.

Beyond gut health, epilactose has shown potential metabolic benefits. In obese mice, Murakami et al. (2015) [26] observed reduced adipose tissue inflammation, increased UCP1 expression in skeletal muscle, improved insulin sensitivity, and modulation of gut microbiota toward *Akkermansia* and *Faecalibacterium*. Such effects are consistent with SCFA-mediated signaling in peripheral tissues.

Given its bifidogenic and butyrogenic properties, high tolerability, and additional metabolic effects, epilactose emerges as a promising functional ingredient for applications in dairy products, nutritional supplements, and specialized formulations targeting infant nutrition, osteoporosis prevention, and metabolic disorders. Potential synergies may be achieved when combined with other prebiotics such as galacto-oligosaccharides (GOS) to broaden microbial stimulation [4].

Epilactose differs from classical prebiotics such as lactulose and GOS, particularly in its fermentation profile, SCFA yield, and tolerability. Comparative studies have shown that epilactose favors butyrate formation, while lactulose and GOS primarily promote acetate [27,28].

Regarding gas production (H_2, CO_2, CH_4), Cardoso et al. (2024) [4] showed that epilactose, lactulose, and raffinose all reduced methane (~4–5-fold compared with control), but epilactose led to greater CO_2 production (~35.7 mmol/L, 4.2-fold higher than lactulose), indicating more intense or distinct fermentation. While clinical data for epilactose are lacking, lactulose is known to cause flatulence at doses >10 g/day and GOS at >15 g/day [29] suggesting that lower doses of epilactose may be advisable to preserve tolerability. Additionally, epilactose promoted the growth of *Acidaminococcus intestini*, *Clostridium baratii*, *Dorea* sp., *Senegalimassilia anaeróbia*, *Bacteroides thetaiotaomicron*, and *Enterococcus faecalis*, depending on the fecal donor's diet [4]. Importantly, the observed CO_2 increase reflects colonic fermentation and is not directly influenced by processing conditions. Nonetheless, applying MEFs during C2E-mediated synthesis could improve the purity and consistency of epilactose, potentially lowering the presence of side substrates that might exacerbate gas formation in sensitive individuals.

From a functional perspective, epilactose combines bifidogenic and butyrogenic properties, exhibiting greater microbial selectivity than lactulose, which can also be utilized

by potentially undesirable bacteria such as *Enterobacteriaceae* [30]. GOS, in contrast, undergo more gradual fermentation and exert a milder effect on butyrogenesis. Structurally, epilactose is a lactose epimer, a feature that may influence its metabolization by specific bifidobacterial strains. These characteristics suggest that epilactose has potential as a prebiotic, although it may cause fermentative side effects when consumed in high doses, similar to other prebiotics. Its prebiotic action is primarily mediated by the selective fermentation previously described, leading to SCFA production with local and systemic benefits.

Furthermore, epilactose increases the paracellular uptake of ions such as Ca^{2+}, Mg^{2+}, and Fe^{2+} by inducing the phosphorylation of the myosin light chain (MLC) at intercellular junctions, via activation of the MLCK and ROCK pathways, as demonstrated by Suzuki et al. (2010) [25]. This effect modulates the epithelial barrier without inducing pathological permeability, as evidenced by the maintenance of the distribution of junction proteins.

In models of metabolic inflammation, Murakami et al. (2015) [26] reported that epilactose reduced macrophage infiltration in adipose tissue and systemic inflammation in obese mice, in addition to increasing UCP1 expression in skeletal muscle, indicating a possible thermogenic action. It is proposed that the generated SCFA, especially butyrate and propionate, act as metabolic signaling in peripheral tissues, promoting increased energy expenditure and improved insulin sensitivity.

Therefore, the mechanisms of epilactose include: (1) modulation of the microbiota with increased beneficial SCFA and reduced toxic metabolites; (2) strengthening of the epithelial barrier and increased mineral absorption; (3) local and systemic anti-inflammatory effects mediated by SCFA; (4) metabolic regulation through intestinal-peripheral tissue signaling (Figure 2).

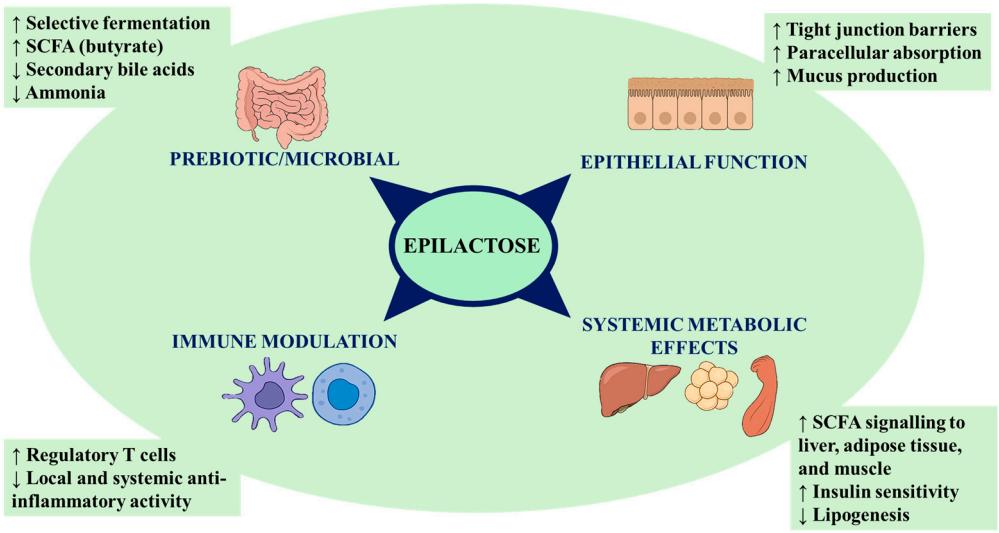


Figure 2. Integrative effects of epilactose on gut microbiota, intestinal barrier, immunity, and metabolic regulation.

Epilactose has potential as a dietary intervention for vulnerable populations. In the elderly, it can favorably modulate the microbiota, increase butyrate production, and stimulate bifidobacteria, contributing to the integrity of the intestinal barrier and the control of chronic inflammation. Its positive effect on calcium and magnesium absorption is also relevant in the prevention of osteoporosis. Nishimukai et al. (2008) [23] reported greater bone density in elderly rats supplemented with epilactose, in addition to a reduction in non-HDL cholesterol, suggesting cardiovascular benefit. Compared to lactulose, traditionally used in geriatrics, epilactose may offer additional advantages, such as less gastrointestinal discomfort and a greater impact on butyrate production and mineral absorption.

In the pediatric population, epilactose is a candidate for incorporation into infant formulas and functional foods, such as FOS and GOS, which mimic human milk oligosaccharides [31]. Although there is no direct evidence yet, it is plausible that infant bifidobacteria, such as *B. infantis*, can metabolize it. In older children, epilactose may act to prevent functional constipation, with the additional benefit of supporting bone growth by facilitating the absorption of calcium and iron.

In patients with inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn's disease, a reduction in butyrate producers and impairment of the epithelial barrier are observed. Prebiotics such as epilactose can restore butyrate production and promote epithelial differentiation, contributing to inflammatory remission [32]. Murakami et al. (2015) [26] also demonstrated that, in mice on a high-fat diet, epilactose reduced obesity, insulin resistance, and systemic inflammation. Extrapolating to humans, epilactose may be useful in individuals with metabolic syndrome, obesity, or type 2 diabetes, by modulating the microbiota (increased *Akkermansia* and *Faecalibacterium*) and promoting SCFA with beneficial metabolic effects. Propionate, for example, regulates appetite and hepatic gluconeogenesis, while butyrate improves insulin sensitivity and energy expenditure.

In terms of formulation, epilactose can be incorporated into functional foods (fermented dairy products, cereal bars, supplements) alone or in combination with other prebiotics. Synergistic strategies, such as association with GOS (bifidogenic effect), may enhance benefits [4].

2.2. Epilactose Synthesis

In addition to chemical processes, prebiotic production is predominantly carried out by microbial fermentation, using especially microorganisms such as *Bifidobacterium* and *Lactobacillus*, or by enzymatic biocatalysis [33]. Among the main prebiotics currently commercialized are fructooligosaccharides (FOS) [34], GOS [35], inulin [36], and lactulose [37], widely applied in functional foods and nutritional supplements.

Lactulose, a functional disaccharide widely used as a prebiotic and laxative agent, is obtained by isomerization of lactose, in which the glucose unit is converted to fructose, resulting in a galactose-fructose bond, with fructose presenting a ketose ring. This process can be carried out by chemical pathways or catalyzed by isomerase enzymes, usually under less specific thermal and alkaline conditions [38]. In contrast, epilactose production requires a highly selective epimerization at the C-2 position of glucose, promoted exclusively by C2E, resulting in the stereochemical inversion of the hydroxyl group at C-2 of glucose, without breaking the glycosidic bond, and formation of the galactose-epiglucose epimer [39]. This fundamental difference in the formation mechanisms gives epilactose greater synthetic complexity, requiring more rigorous operating conditions to ensure efficiency and selectivity. A comparative scheme between the synthesis pathways of lactulose and epilactose is presented in Figure 3, clearly illustrating these structural and enzymatic distinctions.

The reaction catalyzed by C2E occurs at neutral pH (up to 7.5) and temperatures between 50 °C and 85 °C. It uses high concentrations of lactose as substrate, reaching up to 700 g/L. Under these conditions, thermodynamic equilibrium favors only a partial conversion of lactose to epilactose, with typical yields between 25% and 35%. The reversibility of the reaction, together with product inhibition and limited enzyme stability, poses significant challenges for process efficiency [2,19,40,41].

The immobilization of C2E on solid supports, such as the ion-exchange resin Duolite A568, has been explored as a strategy to enhance enzyme thermal stability and enable its use in continuous reaction systems. Sato et al. (2012) [8] demonstrated that C2E from *Rhodothermus marinus*, when immobilized on Duolite A568 and operated in a fixed-bed

reactor at 50 °C, sustained epilactose production for 13 days, reaching a final concentration of 30 g/L from a 100 g/L lactose solution. Similarly, Wang et al. (2016) [42] reported that C2E from *Caldicellulosiruptor saccharolyticus*, immobilized on the same support, retained its catalytic activity over ten consecutive cycles, showing marked improvements in both operational stability and thermal resistance.

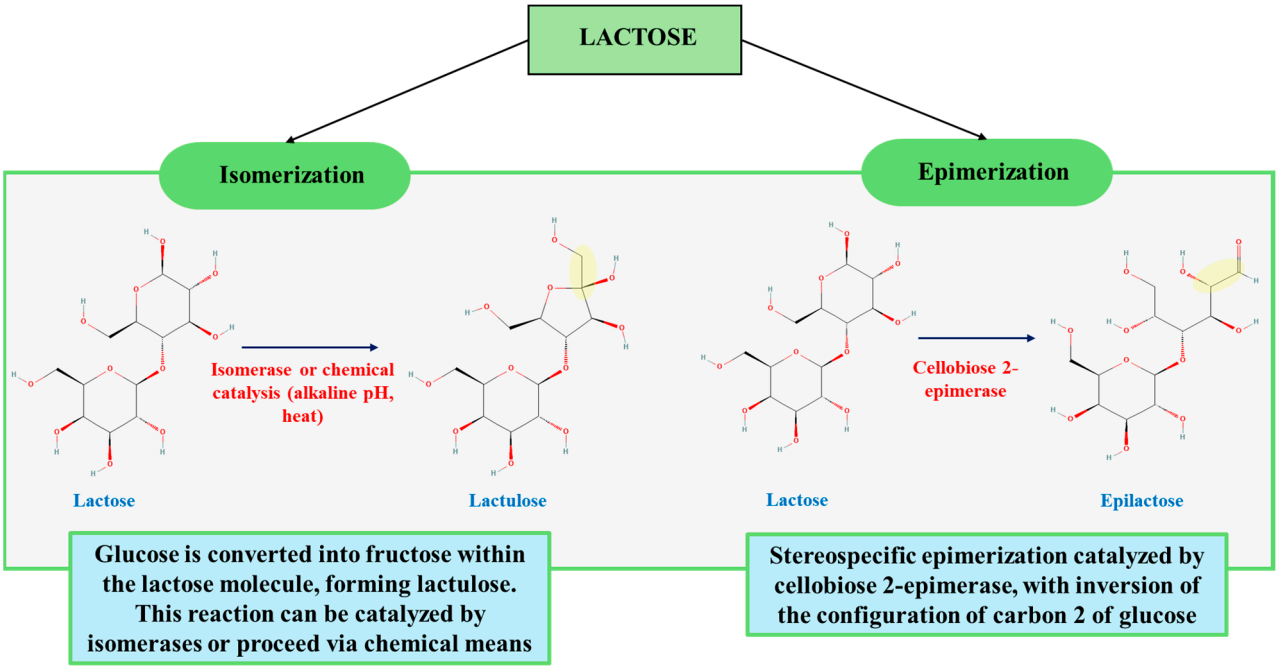


Figure 3. Comparative pathways of lactulose and epilactose synthesis from lactose by isomerization and epimerization.

Another important factor to consider in epilactose production is the choice of substrate. Although pure lactose is ideal due to its well-defined composition, its high-cost limits feasibility for large-scale applications. More economical alternatives include lactose-rich byproducts from the dairy industry, such as whey, ultrafiltration permeate, and demineralized milk. However, these complex matrices often contain metal ions, residual proteins, and organic salts that can interfere with C2E activity and reduce reaction efficiency. For example, divalent cations such as Ca^{2+} and Mg^{2+} may interact with the enzyme or the substrate, altering reaction kinetics and requiring careful optimization of operating conditions [43].

A comprehensive comparison of recent studies on epilactose synthesis using C2E enzymes is presented in Table 1, which highlights key reaction parameters, substrates, enzyme sources, and final yields. The analysis of these data reveals important trends that help elucidate the practical potential and current challenges in the enzymatic production of epilactose [11,19,41].

The majority of C2E applications rely on recombinant enzymes, most commonly expressed in genetically modified hosts such as *Escherichia coli* [19,44], *Bacillus subtilis* [20,45], or *Saccharomyces cerevisiae* [4,10]. These systems enable higher enzyme yields, enhanced stability, and general compatibility with food-grade processes, making them central to the advancement of scalable biocatalytic strategies.

Among the most frequently explored microbial sources of C2E are species from the genus *Caldicellulosiruptor*, such as *C. saccharolyticus* and *C. bescii* [46,47], as well as *Rhodothermus marinus* [20] and *Clostridium* sp. [19]. These thermophilic organisms offer robust enzymes capable of operating at high temperatures (up to 80 °C), which is particularly advantageous for reactions involving highly concentrated lactose solutions (≥300 g/L), as

seen in studies by Liangfei et al. (2022) [2] and Huerta et al. (2024) [46], where epilactose yields exceeded 80 g/L.

It is also evident that the use of more complex substrates, such as milk or whey, can still yield favorable results, provided that the enzymatic system is compatible with the reaction conditions. For example, *Clostridium* sp. TW13 achieved a yield above 32% using expired milk as a substrate [19], whereas enzymes from *Acidobacteriota* sp. were able to synthesize epilactose at refrigeration temperatures directly in milk [48]. These findings highlight promising opportunities for the development of functional dairy products and the valorization of industrial by-products.

From a kinetic perspective, studies consistently report moderate yields ranging from 25% to 35%, with productivity strongly influenced by enzyme thermostability, reaction pH, and enzyme-to-substrate (E/S) ratio. High lactose concentrations (e.g., 700 g/L used by Liangfei et al., 2022 [2]) are beneficial for maximizing space-time yields. However, they require tailored process conditions to ensure solubility and avoid enzyme inactivation.

Selectivity is another strong feature across the examined systems, with minimal lactulose formation reported in most studies (e.g., Cardoso et al., 2021 [10]; Huerta et al., 2025 [41]), supporting the suitability of C2E-catalyzed processes for high-purity epilactose production. Furthermore, some engineered variants, such as the H349N and H356N mutants of cellobiose 2-epimerase from *Caldicellulosiruptor saccharolyticus* (CsCE), offer enhanced control over the epimerization profile and broader operational stability [46].

Table 1. Experimental conditions and yields in epilactose production using cellobiose 2-epimerase.

C2E (Microbial Origin)	Substrate Concentration	Reaction Volume	pH	Assay Temperature (°C)	Reaction Time	Main Results	Ref.
Ruminococcus albus NE1	Lactose, 20 mM (for kinetics); 100 mM (for epilactose synthesis)	3.2 mL (epilactose synthesis); 100 µL (enzyme assay)	7.5 (100 mM sodium phosphate buffer)	30 °C (enzyme assay); 25 °C (epilactose synthesis)	10 min (enzyme assay); overnight (epilactose synthesis)	Epilactose yield from 100 mM lactose reached 38.8%; Km for lactose = 33 mM, kcat/Km = 1.6 $s^{-1} \cdot mM^{-1}$; rCE inactive with maltose and other	[49]
F. johnsoniae (FjCE) and *P. heparinus* (PhCE)	Lactose in UHT milk, ~43 g/L (diluted from 48 g/L)	25 mL (in stirred-tank reactor, STR)	7.0 (10 mM sodium phosphate buffer used to prepare enzyme solution)	8 °C (industrial milk processing condition)	24 h (with maximum production in 9 h)	Epilactose yield: 33.6% (FjCE), 30.5% (PhCE); Final concentration: 14.3 g/L (FjCE), 13.9 g/L (PhCE); No side products detected	[11]
Caldicellulosiruptor saccharolyticus	Lactose, 48.5 g/L (from UHT milk)	25 mL (milk conversion in stirred vessel)	~6.7 (natural milk pH)	8 °C and 50 °C	24 h (at 50 °C); 72 h (at 8 °C)	Epilactose yield: 7.49 g/L (15.5%) at 50 °C; 6.57 g/L (13.6%) at 8 °C	[44]
Clostridium sp. TW13	Lactose, 60 g/L (pure); also tested in whey powder and expired milk	1.5 L (for purification); 1 mL (standard assay)	7.5 (50 mM Tris-HCl buffer)	40 °C (optimum for CsCEase1 activity)	2 h	Conversion rate: 44.3%; Epilactose yield: 26.58 g/L (pure lactose); 29.65% (whey); 32.69% (expired milk); Purity after purification: >98%; Recovery rate: 66.47%	[19]
Caldicellulosiruptor saccharolyticus	Lactose, 100 g/L (ranges of 25–300 g/L tested)	1 mL (batch reactions for kinetic assays)	7.0 (100 mM phosphate buffer)	50 °C (optimal for epilactose production)	4–72 h depending on E/S ratio	Maximum epilactose yield: 0.256 g/g; highest productivity at 40 °C and pH 8.0; epimerization favored at low E/S ratios (1.417×10^{-7} kat/g); no lactulose formed at early stages; high selectivity achievable	[41]
Acidobacteriota bacterium	Lactose, 200 mM (in buffer); 50 g/L (in milk)	0.4 mL (lactose buffer system); 1 mL (milk system)	6.0 (PIPES buffer); milk ~6.7	50 °C (in buffer); 10 °C (in milk, cold catalysis)	2–48 h (buffer); 12 h (milk)	28.5% lactose-to-epilactose conversion at refrigeration temperatures (10 °C)	[48]
Rhodothermus marinus	Lactose, 300 g/L	100 mL (bioconversion system after fed-batch cultivation)	8.0 (Tris-HCl buffer)	80 °C	4 h (max yield at 100 min with 0.5% broth)	Epilactose yield: 29.5%; Productivity: up to 9 g/L/h; Enzyme volumetric activity: 1255 U/mL; Only 0.25% broth needed for 120 kg lactose → 35.4 kg epilactose	[20]
Caldicellulosiruptor saccharolyticus	Lactose, 400 g/L	100 mL	7.0 (100 mM phosphate buffer)	50 °C and 70 °C	48–72 h (epilactose/lactulose synthesis); additional TOS step at 50 °C, pH 4.5	Up to 81.6 g/L epilactose + 22.4 g/L lactulose from unreacted lactose	[46]
Caldicellulosiruptor saccharolyticus	Epilactose 87% purity, final conc. 10 g/L in fermentation medium	40 mL (batch cultures in 70 mL serum bottles)	7.0 (growth medium); dropped to ~5.3–5.6 after fermentation	37 °C (static anaerobic fermentation)	Not applicable (epilactose previously purified)	Epilactose (10 g/L) led to the highest short-chain fatty acid production; butyrate reached 86 mM with the Mediterranean diet	[10]
Thermoanaerobacterium saccharolyticum	Lactose, 300 g/L (from whey powder)	1 L (with whole cell permeabilized B. subtilis)	7.0 (sodium phosphate buffer)	60 °C	1 h	Epilactose yield: 66.9 g/L (22.3%); final purity >98% after purification	[45]
Caldicellulosiruptor saccharolyticus	Lactose, 50 g/L (tested range: 10–270 g/L)	1 mL (standard assay); varied for optimization studies	7.5 (50 mM Tris-HCl buffer)	80 °C (optimum); tested: 50–90 °C	20–120 min	Max epilactose yield: 27% (13.5 g/L); productivity: 9 g/L/h; no lactulose after 20 min; enzyme optimal at 80 °C, pH 7.5; stable up to 60 °C	[10]

Table 1. *Cont.*

C2E (Microbial Origin)	Substrate Concentration	Reaction Volume	pH	Assay Temperature (°C)	Reaction Time	Main Results	Ref.
Caldicellulosiruptor sp.	Lactose, 700 g/L	1 L (biotransformation using whole cells)	8.0–9.0 (optimal); 6.5–7.5 still active	70 °C	2 h	Epilactose yield: 30.4% (210 g/L); productivity: 105 g/L/h	[2]
Caldicellulosiruptor bescii	Lactose, 50 g/L (from cheese whey permeate)	5 L (stirred fermenter)	7.0 (100 mM MOPS buffer)	30 °C (epilactose favored); also tested at 70 °C	24 h	Epilactose yield: 35% (14.9 g/L) at 30 °C; no epilactose formed chemically; reaction is temperature-dependent	[47]

3. Cellobiose 2-Epimerase: Structure, Function, and Applications

To understand the potential for C2E modulation by MEFs, it is essential to understand their structure and mechanism. Cellobiose 2-epimerase (EC 5.1.3.11) is an epimerizing enzyme belonging to the isomerase family, whose main function is the stereochemical conversion of β-1,4 disaccharides, especially lactose, into their epimers, such as epilactose. The three-dimensional structure of the enzyme, elucidated by X-ray crystallography (e.g., PDB ID: 3VTI), presents a TIM (Triosephosphate Isomerase)-barrel (α/β)_8 type fold, with the catalytic site located in the central cavity of the barrel, where substrate recognition and transformation occur [50]. Figure 4 illustrates the three-dimensional structure of C2E, highlighting the TIM-barrel domain and the catalytic site with the main residues involved in epimerization.

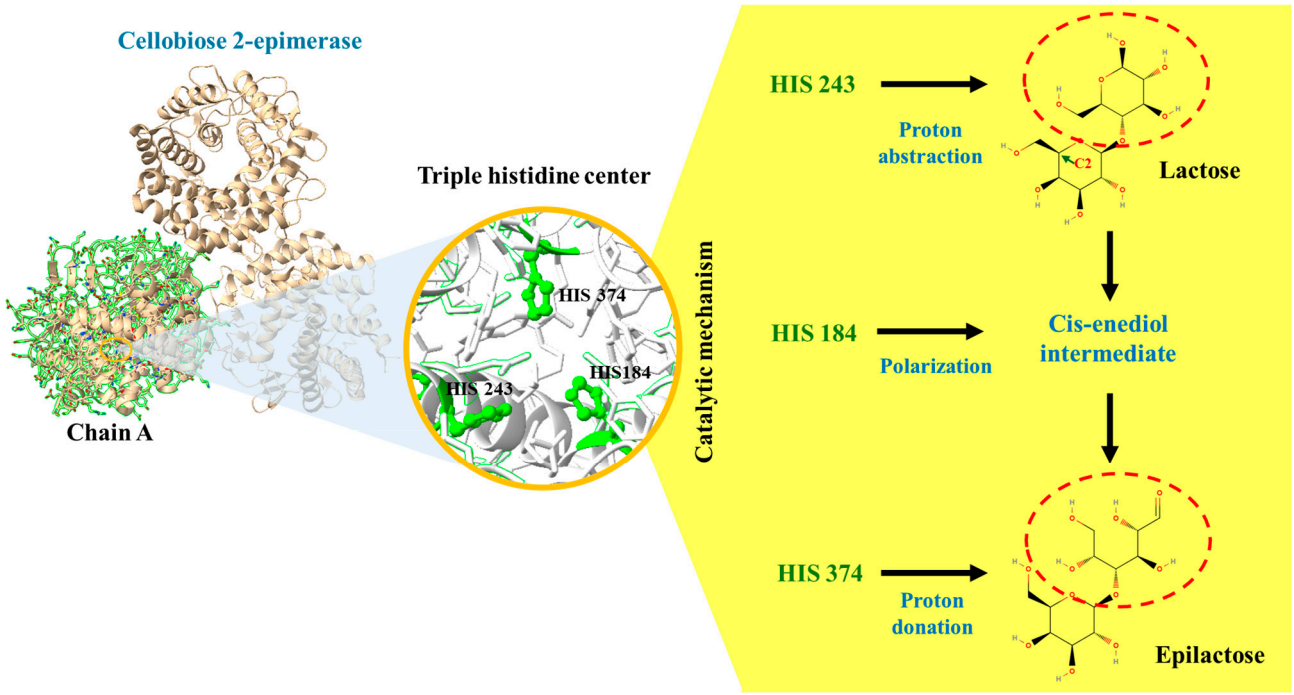


Figure 4. Active site of *Ruminococcus albus* cellobiose 2-epimerase, showing the catalytic histidine triad (His184, His243, His374) and schematic of lactose C-2 epimerization to epilactose, mediated by proton transfer via His243 and His374, with retention of the glycosidic bond.

The active site of C2E from *Ruminococcus albus* contains highly conserved residues that play central roles in the catalysis of epimerization. Among them, His184, His243, and His374 form a triple histidine center responsible for proton transfer during the reaction. Initially, His243 acts as a base, abstracting the axial proton of the hydroxyl group attached to the C-2 carbon of the glucose unit of lactose, leading to the formation of a cis-enediol intermediate. This intermediate is stabilized by interactions with His184, while reprotonation on the opposite face is promoted by His374, resulting in stereochemical inversion at C-2 and formation of epilactose. The residue Glu187 also contributes to the polarization of the hydroxyl group, facilitating proton abstraction. This mechanism occurs without breaking the glycosidic bond and gives the enzyme high selectivity in the stereoselective conversion of disaccharides such as lactose, cellobiose and maltose into their respective epimers: epilactose, epicellobiose and epimaltose [50–52].

Although C2E homologs operate without added metals, divalent cations (e.g., Mn^{2+}/Co^{2+}) have been implicated in stabilizing transition states in related epimerases and may assist substrate positioning in C2E [53]. Additional studies are required to define

cofactor effects under process conditions These cofactors can contribute to the precise positioning of the substrate and the stabilization of the transition state during catalysis, as observed in related enzymes [3,51]. These metals stabilize the transition state of the reaction and contribute to the precise positioning of the substrate in the active site [54]. The presence of the metal cation is essential to maintain the catalytic geometry and favor the efficient transfer of protons, and the absence of these cofactors is associated with a significant loss of enzymatic activity. Although specific data on epilactose are scarce, further studies are needed to clarify its enzymatic parameters and optimize conditions for industrial biosynthesis.

The thermal stability of C2E is influenced by its microbial origin. Mesophilic strains such as *Clostridium* sp. TW13 produce an enzyme with an optimum temperature of 40 °C and a neutral to slightly alkaline pH (7.5), resulting in epilactose yields of over 44% from dairy substrates [19]. On the other hand, strains obtained from thermophilic microorganisms, such as *Caldicellulosiruptor saccharolyticus*, maintain stable catalytic activity up to 80 °C and are tolerant to a wide pH range, from 4.5 to 9.5 [55]. This robustness allows its application in industrial processes that require high temperatures or more extreme environments, reducing the risk of microbial contamination and increasing process efficiency.

Moreover, the use of thermostable enzymes enables direct integration into continuous whey processing flows, which produce a lactose-rich byproduct that remains underutilized and is readily available [2]. The enzymatic conversion of lactose into epilactose, a disaccharide with promising prebiotic properties, represents a viable strategy for valorizing dairy industry wastes and byproducts. The prebiotic potential of epilactose, reflected in its positive impact on health and well-being, supports the growing interest in its commercial-scale production.

C2E has emerged as a key biocatalyst for this purpose, combining catalytic selectivity, a well-defined dependence on metal cofactors, and, in some isoforms, high thermal stability [2,56]. These features make it a suitable enzyme for the synthesis of epilactose from renewable substrates. Nevertheless, its application can be limited by factors such as product inhibition, insufficient operational stability, and suboptimal adaptation to industrial conditions [20]. To address these challenges, strategies for modulating enzyme activity have received increasing attention to enhance biocatalytic performance. Among these, the application of MEFs has emerged as an innovative and still underexplored approach in the context of epimerases. Recent evidence suggests that such physical stimuli can influence enzyme structure, affecting active site accessibility, catalytic dynamics, and substrate affinity. In C2E, this may involve conformational adjustments within its catalytic triad, where His243 is responsible for proton abstraction, His184 for polarization, and His374 for proton donation, as well as nearby charged residues that modulate substrate positioning and stabilize the transition state [57]. Investigating the behavior of C2E under the influence of electric fields opens new possibilities for intensifying epilactose production and contributes to the rational development of more efficient enzymatic systems tailored to the needs of the functional ingredients industry. The following section critically discusses the principles, experimental evidence, and potential applications of enzyme modulation by electric fields, with a focus on disaccharide epimerization.

In addition to the detailed structural knowledge about the catalytic site of C2E, several strategies can be evaluated to modulate its catalytic activity, aiming to increase the efficiency of the conversion of lactose to epilactose. Targeted modifications of amino acids essential for catalysis, such as histidine involved in proton transfer or acidic residues that aid in substrate polarization, can alter the active site environment and improve enzyme performance [56]. Another promising approach involves the application of moderate electric fields (MEFs), which have the potential to influence enzyme conformation, facilitate access to the catalytic

site, and favor critical steps of the mechanism, such as protonation and reprotonation. Despite limited studies involving epimerases to date, these strategies have proven effective for other classes of enzymes [58]. In addition, adjustments in physicochemical conditions, such as pH, temperature, and presence of metal ions, can also be optimized to enhance catalytic activity in industrial environments.

4. Moderate Electric Fields (MEFs): Principles and Effects on Enzymes

The application of electric fields to modulate enzyme activity has been studied as a functional strategy to alter the catalytic performance of enzymes without relying on chemical or genetic modifications. Among the available methods, MEFs stand out for their physical, non-invasive, reversible properties and versatility, allowing the modulation of enzyme activity without modifying the primary structure of the protein or resorting to genetic engineering or covalent immobilization, procedures that generally involve greater technical complexity and operational costs [15]. This approach has advantages in contexts that demand high reproducibility and precise control of enzyme kinetics [15,59].

Enzyme activity is one of the fields of application of MEFs. MEFs have been investigated primarily to enhance enzymatic reaction efficiency, aiming to increase productivity, reduce energy consumption, and optimize operational parameters in biotechnological systems. Although applications remain largely confined to laboratory and pilot scales, studies in food processing, biofuels, and pharmaceutical contexts suggest feasible scalability for industrial deployment [60–62].

Physically, MEFs are characterized by field intensities ranging from 1 to 1000 V/cm, sufficient to induce molecular effects. Depending on the type of microbiological entity, the material, and physical factors (e.g., temperature, pH, ionic strength), MEF treatments may exert effects that lead to membrane permeabilization (often associated with electroporation) [63] or structural changes in macromolecules such as proteins [64]. Despite their low intensity relative to other known electroporation methods such as Pulsed Electric fields (PEF), MEFs can interact with permanent and induced dipoles in enzyme structures, influencing spatial orientation, conformational flexibility, and catalytic site dynamics [16,65]. Field application modes may include continuous (static), sinusoidal (oscillating), or pulsed (intermittent), and operate at frequencies from 10 Hz to MHz ranges. The choice of regime influences specific interactions, such as dipole alignment, conformational changes, or microenvironmental modulation [66,67].

The mechanism of MEF action involves interaction with electrically sensitive regions of proteins, particularly charged residues (e.g., Asp, Glu, Arg, Lys), polar side chains, and metal cofactors. These interactions generate dipolar torques and polarization forces, potentially inducing conformational rearrangements, especially in flexible regions such as catalytic loops, interdomain interfaces, or peripheral domains [68]. In the case of C2E, several structural features suggest responsiveness to MEFs. The enzyme contains divalent metal ion cofactors (Mn^{2+} or Co^{2+}) essential for catalysis, as well as flexible catalytic loops near the active site. The catalytic histidine triad (His243, His184, His374) is positioned within this environment, where precise orientation is required for proton abstraction, polarization of the cis-enediol intermediate, and proton donation, respectively. Nearby charged residues, such as Asp189, Glu243, and His247, contribute to metal coordination and transition state stabilization, making them susceptible to field-induced perturbations [59]. MEFs could modulate the positioning of the catalytic histidine, shift metal ion coordinates, and alter the electrostatic landscape of the active site, thereby improving substrate alignment and lowering activation energy barriers. Oscillatory fields may further promote cooperative domain movements, enhancing catalytic efficiency [69–71].

Table 2 summarizes key studies demonstrating MEF effects on enzymatic systems. For example, Queirós et al. (2023) [60] reported a 40% increase in pectinase activity under 2–20 kHz MEFs at 20 °C, with a corresponding decrease in activation energy from 9.2 to 6.6 kJ/mol. Li et al. (2022) [72] observed a 23% enhancement in α-amylase activity during cornstarch hydrolysis (2.5 V/cm, 50 Hz), associated with an increased α-helix content and the flexibility of peripheral regions. Lu et al. (2022) [73] described the up to 122% activation of β-glucosidase under pulsed 15 kV/cm fields, with reductions in activation enthalpy and entropy, confirming the structural reorganization capacity of MEFs.

While inactivation effects have been reported for oxidative enzymes such as peroxidase (POD) and polyphenol oxidase PPO [74,75], other studies indicate potential for catalytic enhancement. For instance, Brochier et al. (2019) [61] showed peroxidase activation at 10 Hz and 60 °C, and Durham and Sastry (2020) [15] demonstrated increased cellulase activity under 8–12 V/cm at suboptimal temperatures, attributed to electrophoretic chain movement.

Structural studies on other enzymes have shown that moderate electric fields can induce reversible alterations in α-helical content, which may modulate catalytic properties [76]. However, no experimental evidence is currently available to confirm whether such reversibility occurs in C2E. Therefore, any potential for MEFs to induce reversible α-helical changes in C2E remains hypothetical and is extrapolated from observations in related enzymatic systems. Li et al. (2022) [77] further suggested asynchronous domain oscillations as a mechanism of enzyme activation.

Table 2. Applications of moderate electric fields (MEFs) and pulsed electric fields (PEF) in enzyme processing and food preservation.

Application	Enzyme	Reaction Medium	MEFs Configuration	MEFs Parameters	Assay Temperature (°C)	Effect on Activity	Structural Insights	Ref.
Non-thermal pasteurization of sugarcane juice	Peroxidase (POD)	Sugarcane juice	MEFs and ohmic heating	10 to 10^5 Hz, sinusoidal waveform, 0–16.7 V/cm	60–80	Activation (10 Hz, 60 °C); Inactivation (≥50 Hz, 80 °C)	Browning; loss of phenolic compounds	[61]
Hydrolysis of corn starch (native and gelatinized); starch modification via MEF	α-amilase	Corn starch suspensions (native and fully gelatinized)	MEFs, alternating current	2.5 V/cm, 50 Hz, up to 120 min; with or without Ca^{2+} (0–50 mM)	40–70	Activity increased by 23.3% with low-intensity MEF (2.5 V/cm); effects depend on intensity, frequency, Ca^{2+} ions, temperature, and time	Structural changes (increase in α-helix and β-sheet); changes in fluorescence; greater hydrolysis in gelatinized vs. native starch	[72]
Blanching and pasteurization of mushrooms with higher efficiency	Tyrosinase (*Agaricus bisporus*)	50 mM phosphate-buffer solution, pH 6.5	MEFs with ohmic heating (OH), alternating current (AC)	25, 30, and 35 V/cm; 60 Hz; up to 15 min	50–58	OH reduced inactivation time (D-values from 42.14 min to 6.44 min at 58 °C); synergistic effect between electric field and temperature	Suggested denaturation by polarization of the metallic active site; structural alteration facilitated by the electric field	[78]
Efficient pasteurization of orange juice	Peroxidase	Orange juice	Ohmic heating (AC), continuous vs. intermittent	15 V/cm; continuous vs. intermittent	60	More efficient inactivation under continuous treatment	Electric field improves heat and mass transfer	[79]
Non-thermal preservation of grape juice	Not applicable (enzyme not studied)	Grape juice	MEFs (AC)	15.6, 18.8, 21.9 V/cm; 50 Hz; 5, 10, 15 min	25	Not applicable (emphasis on physicochemical and microbiological parameters)	Reduction in pH; increase in turbidity and conductivity; degradation of phenolic compounds and vitamin C; microbial reduction up to 3.43 $\log_{10}$	[80]
Selective inactivation of enzymes in tomato pulp	Pectin methyl esterase (PME) and Polygalacturonase (PG)	Tomato pulp	MEFs	Frequência from 0 Hz (DC) to 1 MHz, 0.4 V/cm, 300 s	-	PME: significant inactivation up to 26% at 1–60 Hz; PG	Increased molecular motion at low frequencies	[81]
Hydrolysis of pre-gelatinized corn starch via MEF	α-amylase	Corn starch suspension	MEFs (AC)	2.5 and 5 V/cm, 50 Hz, 30 min	Not controlled	Higher activity at 2.5 V/cm; inactivation at 5 V/cm; increased content of reducing sugars (up to 0.92 mg/mL)	Destruction of semicrystalline structure; formation of new crystals aligned by the field	[77]
Inactivation of peroxidase in sugarcane juice	Peroxidase (POD)	Sugarcane juice (liquid, solid, and concentrated fractions)	Ohmic heating and MEFs	7.8 V/cm, 60 Hz, 75 °C, 25 min	75	Inactivation: 78% (liquid), 100% (solid), 96% (concentrated); OH more effective than conventional heating (CH)	Sugar-stabilizing effect; influence on isoenzymes and fraction behavior; thermal effect enhanced by electric current	[82]
Inactivation of POD and PPO and preservation of compounds	Peroxidase (POD) and Polyphenol oxidase (PPO)	Sugarcane juice	Ohmic heating and MEFs	3.57 to 4.39 V/cm, 25 V applied, 60–80 °C, up to 12 min	60 to 80	POD: greater inactivation with OH at 80 °C (up to 72%); PPO: almost completely inactivated	Preservation of phenolics in sugarcane juice; possible activation of POD at 60 °C; variations in thermal inactivation phase; improved stability under MEF	[13]
Increased enzymatic saccharification of lignocellulose in a bioreactor	Cellulases (endoglucanase, cellobiohydrolase, and β-glucosidase)	Pre-treated rice straw (1% NaOH)	Pulsed electric field	0.12 V/cm, 48 °C, 96 h, electrode change every 6 h	48	Improved conversion efficiency (22.8%); 32.6% increase in saccharification	Greater enzyme mobility and adsorption to cellulose surface; possible denaturation under high field strengths	[83]

Table 2. *Cont.*

Application	Enzyme	Reaction Medium	MEFs Configuration	MEFs Parameters	Assay Temperature (°C)	Effect on Activity	Structural Insights	Ref.
Microbial and α-amylase inactivation in oat-based plant beverage with pea protein	α-amylase (Bacillus subtilis)	Non-sterilized plant beverage (oat + pea protein)	Pulsed electric field (PEF)	8.2–10.4 kV/cm, 77–244 kJ/L, 25 s, preheating 30–45 °C	Up to 85	Inactivation up to 89.2%; effect dependent on energy and preheating	Synergistic effect with preheating; limited thermal stability of the enzyme	[84]
Non-thermal inactivation of oxidative enzymes in grape juice	Polyphenol oxidase (PPO) and Peroxidase (POD)	Fresh grape juice (red grape, seedless)	MEFs	82 to 87 V/cm, 65 to 75 °C, 10 min	65 to 75	PPO: complete inactivation in 6–8 min (87 V/cm, 75 °C); POD: complete inactivation in 8–10 min (87 V/cm, 75 °C)	Molecular motion simulation shows increased kinetic energy of enzymes with MEF; field-temperature synergistic effect	[75]
Enhance enzymatic cellulose hydrolysis at suboptimal temperatures	Cellulases (Cel7A, Cel6A, Cel7B, etc.) from *Trichoderma reesei*	Whatman No. 1 filter paper disks (cellulosic substrate model)	MEFs	8–12 V/cm, 50 Hz, 30–55 °C, up to 24 h	30 to 55	Increased activity below the optimum (up to +264% in k_1 at 30 °C); activity loss above 50 °C	Molecular motion simulations show increased kinetic energy of enzymes under MEF; synergistic field-temperature effect	[15]
In situ control of α-amylase activity by MEF frequency	α-amylase	Diluted potato starch solution in acetate buffer (pH 5.0)	MEFs	1 V/cm; 1 Hz–1 MHz, 60 °C, 25 s	60	Up to 41% increase in in situ activity (1–60 Hz); mild inhibition at high frequencies	Transition from oscillatory to rotational motion below 60 Hz; possible increase in enzyme–substrate collisions	[66]
Pasteurization and PPO inactivation in mango pulp	Polyphenol oxidase (PPO)	Mango pulp	Ohmic heating and MEFs	15–20 V/cm, 72 °C, 15–120 s	72	Up to 95.7% inactivation with 15 s (PPO); partial reactivation possible between 45 and 60 s	Increase in soluble fiber and viscosity; electroporation and enzyme structural modification	[85]
Non-thermal inactivation of PPO in apple juice	Polyphenol oxidase (PPO)	Fresh apple juice (Red Delicious)	MEFs	85–110 V/cm, 50 °C (on-off); 92.5–98 V/cm, 50–65 °C (constant)	50 to 65	Inactivation up to 89% (65 °C); inactivation rate doubled compared to thermal control (50 °C)	Synergistic field-temperature effect; lower specific energy required with on-off mode	[74]
Green wine clarification with PEC under MEF	Pectinase (PEC)	Green wine (simulated and real)	MEFs	7 V/cm, 20 kHz, 15–35 °C	15–35	Activity increased up to 29% (15 °C) and 21% (20 °C); lower activity above 25 °C	42% reduction in pectin content in must at 20 °C with MEF; lower thermal stability	[60]
Internal heating and PPO and POD inactivation in kiwi juice	Polyphenol oxidase (PPO) and Peroxidase (POD)	Fresh kiwi juice (Actinidia chinensis)	Induced electric field (IEF) by alternating magnetic field	1800 V *, 6 mL/min, 60 kHz, 7.7 s	65.4	PPO: 94.8% inactivation; POD: 92.7% inactivation	Lower loss of phenolics (7.1%) and flavonoids (2.7%); better color preservation; lower browning index	[86]
Enzymatic hydrolysis of soy isoflavone glycosides assisted by PEF	β-glucosidase (β-GLU)	Soy isoflavone glycosides in acetate buffer (pH 5.0)	Pulsed electric field (PEF)	5–25 kV/cm; 6–10 pulses; 1000–3000 Hz; 2 µs pulse width	20–23	Up to 122.1% activity increase at 15 kV/cm; α-helix increase	Ea, ΔH, ΔS reduction, and ΔS; increase in α-helix content; conformational changes (CD and fluorescence)	[73]
Preservation of polyphenols and increased bioavailability in fruits and vegetables	PPO/POD	Various fruit juices and pulps (e.g., jujube juice, kiwi, apple, etc.)	Pulsed electric fields (PEF), cold plasma (CP), and high-pressure processing (HPP) (as comparative reference)	20–65 kHz (CP), 25–35 kV/cm (PEF), 300–600 MPa (HPP); duration ranging from 1 to 30 min	60	Reduction or inactivation of PPO/POD by structural disruption or oxidative stress	Increased total phenolic content (TPC); cell membrane rupture; induction of plant stress	[87]

* Values expressed only as potential difference (V) in the original source; it was not possible to calculate the electric field (V/cm).

In the case of C2E, MEFs offer a high-potential strategy for catalytic enhancement. This enzyme catalyzes lactose epimerization via a histidine triad (His243, His374, His184) in a TIM barrel structure. MEFs may fine-tune this active site by inducing conformational micro adjustments and altering the pKa of catalytic residues, promoting more efficient catalysis [51,88].

Given its structural flexibility and metal cofactor dependency, C2E is comparable to other MEF-responsive enzymes such as β-glucosidase, α-amylase, and cellulase. Evidence suggests that MEFs may promote: (i) conformational adjustments in catalytic loops, (ii) electrostatic modulation of the active site and pKa shifts, and (iii) dynamic flexibility of structural domains, enhancing substrate access and turnover.

While these hypotheses await experimental validation, they are mechanistically consistent with MEFs effects observed in related systems. MEFs have also been applied in food matrices for ingredients compounds extraction [89–91], protein modification [92–94], and emulsification control [95–97], where effects include protein unfolding, exposure of hydrophobic residues, and modulation of rheological properties.

Although no direct studies have yet investigated the electrical effects on C2E, the molecular architecture of the enzyme suggests a plausible basis for such interactions. Figure 5 presents a conceptual model illustrating potential MEFs-induced structural effects, including dipole reorganization, catalytic triad realignment, increased domain flexibility, and reduced activation energy. These proposed mechanisms offer a theoretical foundation for future experimental and computational studies aimed at enhancing epilactose production through enzyme modulation.

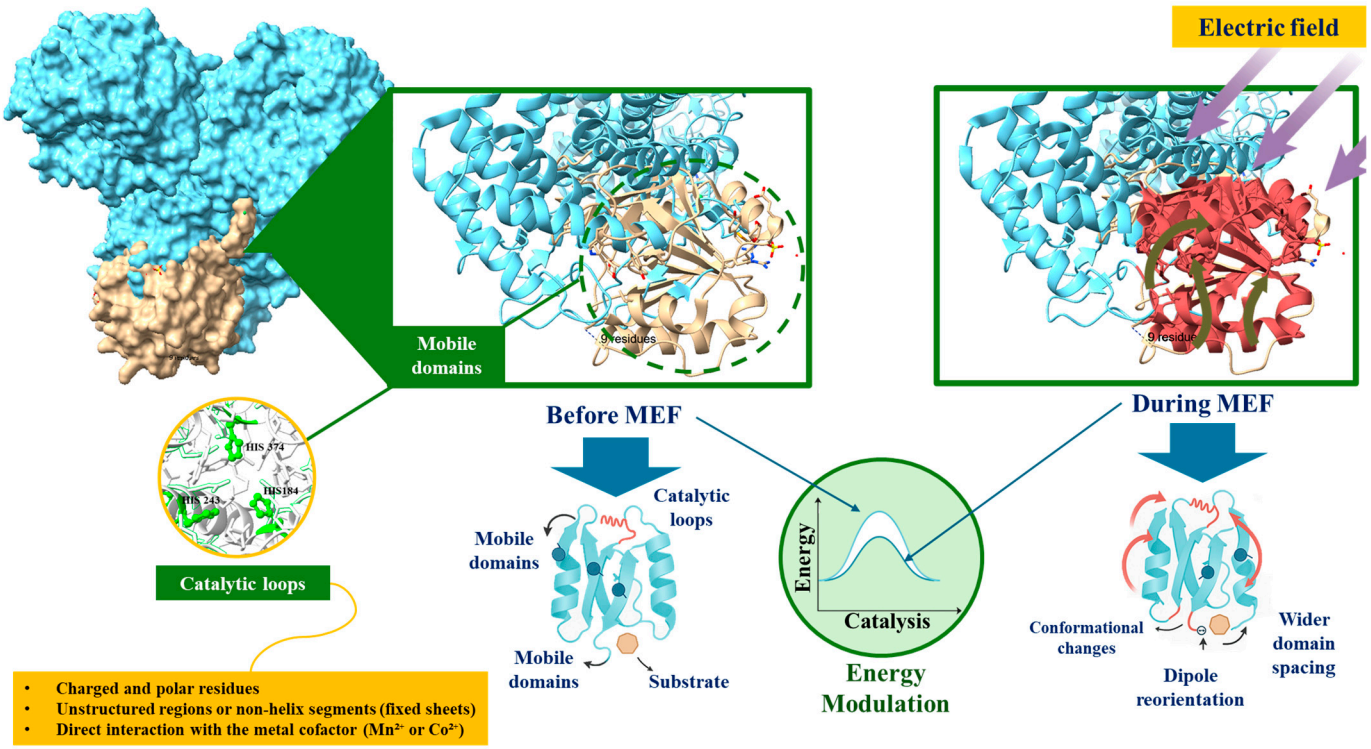


Figure 5. Proposed conceptual model of MEFs-induced conformational changes in C2E and their catalytic implications.

5. Perspectives on the Use of Moderate Electric Fields (MEFs) for Enzymatic Modulation

The use of MEFs to modulate enzymatic properties represents a promising yet still emerging strategy for enhancing biocatalytic processes. Although recent studies have shed

light on the effects of MEFs in specific enzymatic systems [69], industrial adoption remains limited, and the underlying mechanisms are not yet fully understood [15].

A major challenge in advancing this approach lies in the lack of standardized experimental protocols. Variations in key parameters such as field strength, waveform, frequency, temperature (i.e., ohmic heating effect) and exposure time hinder reproducibility and complicate comparisons across studies. This methodological inconsistency also makes it difficult to establish clear mechanistic relationships between MEFs conditions and enzymatic performance [98].

Expanding MEFs research beyond traditional targets such as hydrolases and oxidoreductases, and including less-studied enzyme classes like lyases, ligases, and transferases, may significantly broaden its technological relevance [99]. C2E, for example, is a suitable candidate for such studies due to its well-characterized active site and cofactor dependencies, which enable detailed investigation of MEF responsiveness and selectivity [47].

Combining MEFs application with high-throughput screening and computational modeling offers a powerful framework for optimizing stimulation parameters and uncovering enzyme-specific activation profiles. Such integration could accelerate the rational development of MEF-assisted biocatalytic systems tailored for industrial use [99].

The combination of MEFs with other non-thermal processing techniques, such as ultrasound, high-pressure processing, or cold plasma, has the potential to produce synergistic improvements in enzyme activity, stability, and substrate accessibility. These hybrid strategies may help overcome key challenges in process scale-up, including uneven field distribution, energy efficiency, and system integration [100].

A critical step toward industrial application is the validation of MEF effectiveness in complex reaction media, such as food matrices, fermentation broths, or biorefinery effluents [101]. For example, in lactose-rich by-products like whey, in situ activation of C2E using MEFs could enable on-site epilactose production, reducing downstream processing requirements and aligning with circular economy and resource valorization principles [102].

Another promising direction involves using real-time process control. Spectroscopic methods to monitor changes in substrate levels, product accumulation, or reaction kinetics may offer the opportunity to fine-tune enzymatic activity. This type of adaptive control would be particularly beneficial in continuous bioprocessing systems [103]. To support industrial adoption, rigorous techno-economic evaluations and standardized life cycle assessments (LCAs) are needed. These should address factors such as energy consumption, compatibility with existing equipment, regulatory compliance, and impacts on product yield and quality [104].

Although our foundational understanding of MEF-induced enzymatic modulation has advanced, translating this knowledge into deployable technologies requires interdisciplinary collaboration across enzymology, process engineering, computational modeling, and industrial biotechnology [105].

Specifically, for C2E and the enzymatic production of epilactose, MEFs represent a compelling approach to overcome current limitations in conversion efficiency and process intensification. Continued research in this area has the potential to drive the development of more sustainable, controllable, and efficient biocatalytic systems across the food, pharmaceutical, and chemical sectors.

6. Conclusions

The modulation of enzymatic activity by MEFs represents a compelling approach for enhancing biocatalytic processes, particularly in systems that demand fine structural and kinetic control. In the case of C2E, structural features such as flexible catalytic loops, metal ion dependency, and strategically positioned charged residues suggest a high po-

tential for responsiveness to MEF-induced effects. These may include conformational adjustments, dipole reorientation, and electrostatic tuning of the active site, which could potentially improve the efficiency of lactose-to-epilactose conversion. Although the application of MEFs to epimerases remains largely unexplored, positive outcomes observed in other enzyme classes reinforce the viability of this strategy. The ability to modulate enzyme function in a reversible, real-time, and non-invasive manner also aligns with the demands of modern bioprocesses, particularly those operating under continuous or thermally constrained conditions.

Advancing this approach will require multidisciplinary efforts combining structural enzymology, biophysics, and process engineering to validate and optimize MEF-based catalytic modulation for industrial use. Integrating complementary strategies, such as computational modeling to predict MEF-induced structural and functional changes, targeted laboratory-scale experiments to validate these predictions and define optimal field parameters, and pilot-scale trials to assess process performance under industrial conditions, could accelerate the translation of MEF-assisted C2E into practical applications. This progression would also support the sustainable valorization of lactose-rich by-products, positioning epilactose as a valuable prebiotic ingredient in innovative food and nutraceutical products.

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