

Non-Thermal Green Processing Technologies (HPP, PEF, and Ultrasound) for Milk Preservation: Impact on Bioactive Milk Proteins and Enzymatic Kinetics

Santoshkumar M. Dapkekar

Department of Dairy science, Sambhajirao Kendre College, Jalkot, Dist- Latur-413532, Maharashtra, India

Corresponding Author: dapkekar1976@gmail.com

Abstract

Consumer demand for minimally processed dairy products with intact nutritional and functional characteristics has accelerated research into non-thermal 'green' preservation technologies as alternatives to conventional thermal pasteurization. This review examines three of the most extensively studied non-thermal technologies for milk preservation — high-pressure processing (HPP), pulsed electric field (PEF) processing, and power ultrasound — with specific focus on their impact on bioactive milk proteins (immunoglobulins, lactoferrin, lactoperoxidase, and related whey proteins) and on the enzymatic inactivation kinetics that underpin process validation and safety assurance. HPP disrupts non-covalent interactions within casein micelles and can denature pressure-sensitive whey proteins such as β -lactoglobulin at pressures above approximately 400 MPa, while comparatively preserving lactoferrin and immunoglobulin structure at moderate pressure-time combinations. PEF acts primarily through electroporation of microbial membranes and achieves microbial and partial enzymatic inactivation with minimal bulk heating, generally retaining more native protein structure than thermal treatment. Ultrasound-induced acoustic cavitation can physically remodel fat globules and protein aggregates, but substantial enzyme inactivation typically requires combination with mild heat (thermosonication). We further compare the kinetic modeling frameworks — first-order (D/z -value), Weibull, quasi-chemical, and PEF-specific empirical models — used to describe enzyme inactivation across these technologies, highlighting where classical first-order assumptions break down and non-linear models are required. The review concludes with a discussion of regulatory status, industrial scale-up barriers, and research priorities for translating laboratory-scale non-thermal processing into validated, commercially deployable milk preservation systems.

Keywords: *non-thermal processing; high-pressure processing; pulsed electric field; ultrasound; milk proteins; enzyme inactivation kinetics; dairy science*

1. Introduction

Thermal pasteurization has been the cornerstone of milk safety assurance for over a century, reliably inactivating pathogenic microorganisms and indicator enzymes such as alkaline phosphatase (ALP). However, thermal treatment also denatures heat-labile bioactive proteins — including immunoglobulins, lactoferrin, lysozyme, and native lactoperoxidase (LPO) — that contribute to the antimicrobial, immunomodulatory, and antioxidant functionality associated with raw and minimally processed milk [1]. This trade-off has motivated sustained interest in non-thermal 'green' processing technologies that can achieve equivalent microbial safety while better preserving these functional components.

High-pressure processing (HPP), pulsed electric field (PEF) processing, and power ultrasound are the three non-thermal technologies most extensively investigated for milk preservation [2], [3]. Each achieves microbial inactivation through a fundamentally different physical mechanism — hydrostatic pressure, electroporation, and acoustic cavitation, respectively — and each interacts differently with milk's protein and enzyme systems. Because regulatory validation of any pasteurization-equivalent process depends on well-characterized inactivation kinetics for target enzymes and pathogens, understanding how these three technologies affect not only the extent but also the kinetic pattern of protein and enzyme inactivation is essential for both food safety assurance and functional quality optimization.

2. High-Pressure Processing (HPP)

2.1 Mechanism

HPP subjects milk, typically packaged, to hydrostatic pressures of 400–600 MPa for holding times of roughly 1–10 minutes at near-ambient or mildly elevated temperatures [2]. Unlike thermal treatment, HPP predominantly affects non-covalent interactions — hydrogen bonds, hydrophobic interactions, and electrostatic bonds — while leaving covalent bonds largely intact. This selectivity underlies HPP's ability to inactivate microorganisms and some enzymes while preserving small molecules, vitamins, and flavor compounds relatively unchanged.

2.2 Effects on Caseins and Whey Proteins

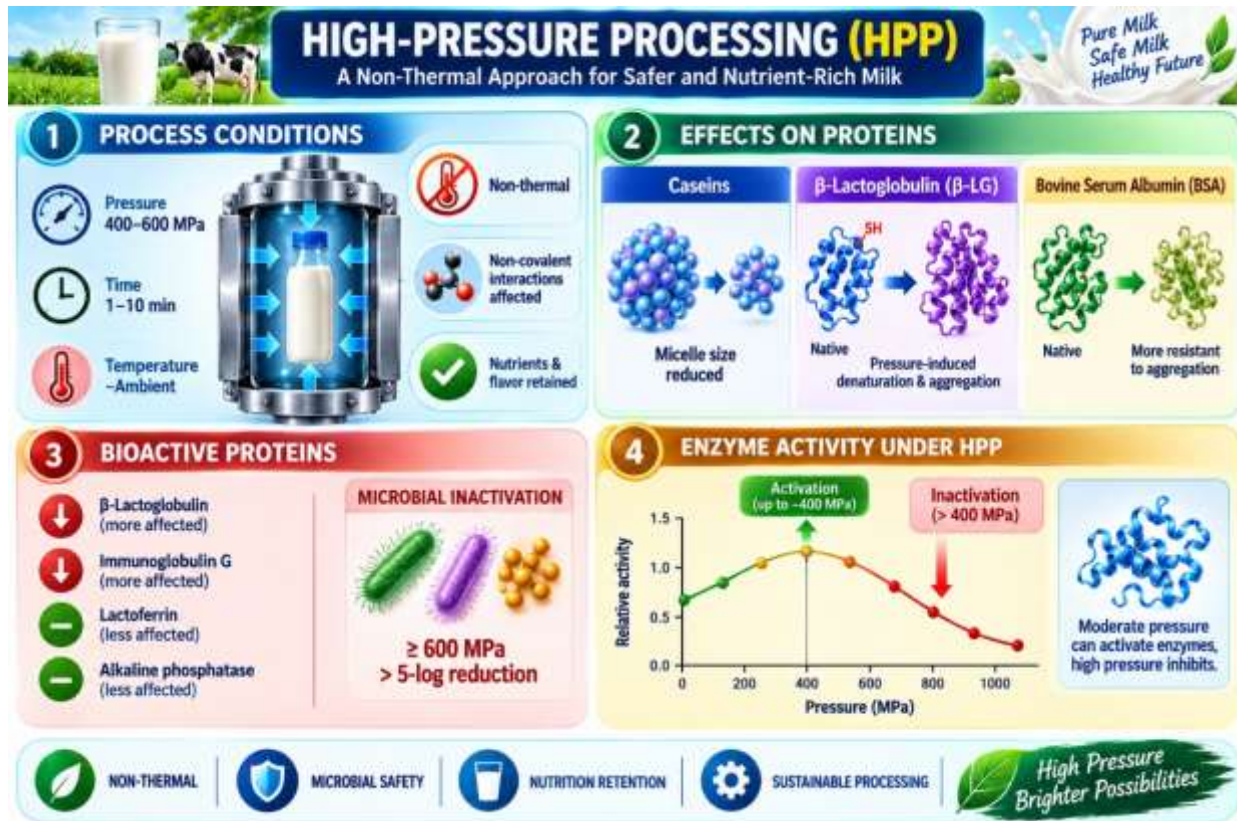
Pressure treatment reduces the average size of casein micelles, primarily through disruption of the hydrophobic and electrostatic interactions that stabilize micellar structure, an effect that can be modulated by lactose content and processing time [2]. Among whey proteins, β -lactoglobulin is particularly pressure-sensitive: its single free thiol group becomes exposed under pressure, promoting thiol-disulfide exchange reactions and aggregation, with denaturation becoming pronounced above approximately 400 MPa. Bovine serum albumin (BSA), by contrast, shows significant secondary-structure change at higher pressures ($\sim$ 800 MPa), but its fifteen disulfide bonds confer resistance to irreversible aggregation up to very high pressures ($\sim$ 1270 MPa), and pressure-induced secondary-structure changes in whey proteins are often at least partially reversible upon decompression.

2.3 Effects on Bioactive Proteins

A systematic review of HPP operating parameters found that pressures of 600 MPa or greater are typically required to achieve more than 5-log reductions in key pathogens such as *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus* in bovine milk, but such pressures often cause considerable denaturation of β -lactoglobulin and immunoglobulin G, while lactoferrin and alkaline phosphatase are comparatively less affected [1]. This finding highlights a central tension in HPP process design: the pressure-time combinations needed for robust microbial safety do not always align with those that best preserve specific bioactive proteins, and most published studies have examined microbial safety and bioactive protein preservation separately rather than jointly, leaving the identification of a single pressure-time 'sweet spot' incomplete.

2.4 Enzyme Activity and Inactivation Kinetics under HPP

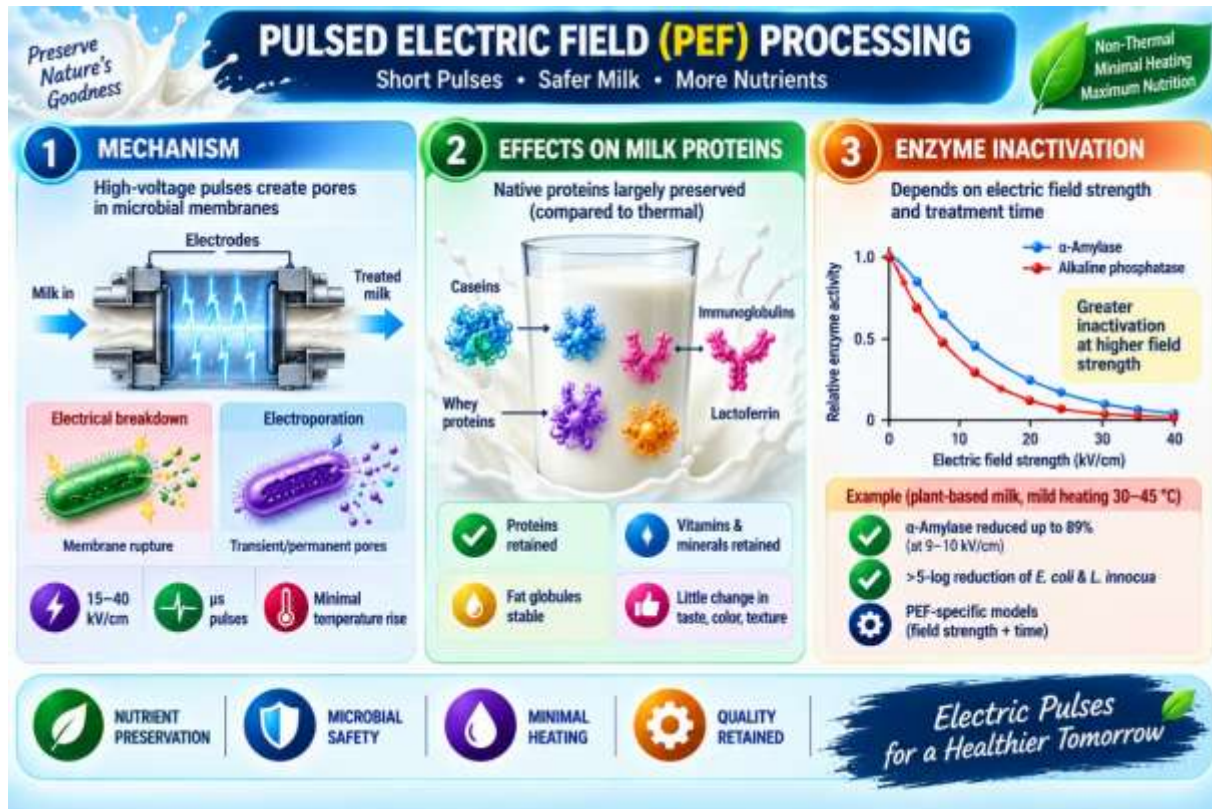
HPP's effect on milk enzymes is notably non-monotonic with respect to pressure. Moderate pressures (up to approximately 400 MPa) combined with mild heating have, in several studies, been reported to activate or stabilize certain milk enzymes rather than inactivate them — with pressures of 350–400 MPa sustained for extended holding times (on the order of 100 minutes) reported to increase activity of some milk enzymes by up to 140% — whereas higher pressures progressively inhibit enzyme activity, consistent with protein unfolding and active-site disruption at more extreme conditions. This pressure-dependent activation-then-inactivation behavior distinguishes HPP from thermal and, to a lesser extent, PEF treatment, and has direct implications for kinetic modeling: because inactivation rate is not monotonic across the full pressure range, single-parameter first-order models fit only within restricted pressure windows, and more flexible kinetic frameworks (Section 5) are often needed to describe the full behavior.



3. Pulsed Electric Field (PEF) Processing

3.1 Mechanism

PEF applies short, high-voltage electrical pulses (typically 15–40 kV/cm, microsecond-scale pulse widths) to milk flowing between electrodes, inducing electroporation of microbial cell membranes. Two mechanisms are generally invoked: electrical breakdown, where the applied field exceeds the critical field strength of the microbial membrane and causes direct membrane rupture, and electroporation, where induced transmembrane voltage creates transient or permanent pores in the membrane [4]. Because PEF is applied for microsecond-scale pulses with limited net energy input, bulk product temperature increases are typically modest, allowing PEF to be classified as a genuinely non-thermal or minimally thermal technology relative to HPP and ultrasound.



3.2 Effects on Milk Proteins

Compared with thermal pasteurization, PEF-treated milk shows substantial retention of major nutrients including proteins, minerals, and vitamins, with minimal effect on texture, taste, and color, and with fat globule structure remaining largely stable [5]. Functional property studies have similarly reported that PEF processing of bovine milk causes only limited changes to protein functionality relative to conventional heat treatment [6], supporting PEF's positioning as a technology capable of preserving native protein structure — including immunoglobulins and other heat-labile bioactive proteins — more effectively than thermal pasteurization.

3.3 Enzyme Inactivation Kinetics under PEF

PEF can inactivate several indigenous milk enzymes, but the extent of inactivation is strongly dependent on field strength, treatment time, and specific energy input rather than on temperature alone. Studies of bovine whole milk treated by PEF report measurable reductions in both microbial load and select enzyme activities, with the degree of enzyme inactivation generally lower than that achieved by equivalent thermal processing at comparable microbial log-reductions [7]. In blended plant-based milk alternatives, PEF combined with mild preheating (30–45 °C) at moderate field strengths (9–10 kV/cm) has been shown to reduce α -amylase activity by up to 89% while achieving at least 5-log reductions of *E. coli* and *Listeria innocua* surrogates, illustrating that PEF enzyme inactivation kinetics are highly sensitive to the combination of electrical and thermal inputs rather than either parameter alone [8]. Because PEF inactivation depends jointly on field strength and treatment time in a manner not well captured by classical thermal D/z-value kinetics, PEF-specific empirical models have been developed (Section 5) that treat electric field strength as a primary kinetic variable analogous to temperature in thermal models.

4. Ultrasound (Power Ultrasound and Thermo-sonication)

4.1 Mechanism

Power ultrasound (typically 20–100 kHz at high intensity) generates acoustic cavitation: the formation, growth, and violent collapse of microbubbles in the liquid medium. Bubble collapse produces localized transient temperatures and pressures, intense shear forces, and free-radical formation, all of which can disrupt microbial cell membranes and physically alter macromolecular structures without necessarily requiring bulk heating [9].

4.2 Effects on Milk Proteins and Structure

Ultrasonic treatment can homogenize protein aggregates and reduce fat globule size in dairy systems, producing structural changes distinct from those caused by conventional high-pressure homogenization [10]. Because whey proteins are thermolabile, ultrasonic treatment applied without significant heat generation is often proposed as an alternative processing route that avoids whey protein precipitation associated with thermal denaturation [11].

4.3 Enzyme Inactivation Kinetics under Ultrasound

A recurring finding across the ultrasound literature is that ultrasound alone, applied without concurrent heat generation, has little effect on indigenous milk enzymes. In a controlled comparison of continuous-flow high-intensity ultrasound with and without heat generation, alkaline phosphatase, γ -glutamyltranspeptidase, and lactoperoxidase activities were essentially unaffected by ultrasound alone, whereas ultrasound combined with heat produced the greatest enzyme and whey protein denaturation, with clear synergistic inactivation observed at 61, 70, and 75.5 °C for alkaline phosphatase, γ -glutamyltranspeptidase, and lactoperoxidase, respectively [12]. This pattern has been confirmed in subsequent reviews, which similarly conclude that inactivation of alkaline phosphatase and lactoperoxidase during ultrasonic pasteurization of raw milk requires combination with heat treatment (thermosonication) rather than ultrasound alone [11]. From a kinetic standpoint, this means ultrasound-alone treatments often show negligible or very slow first-order inactivation rates for these marker enzymes, while thermosonication produces inactivation kinetics that are typically faster than either heat or ultrasound applied independently — a synergistic rather than additive effect that complicates straightforward kinetic modeling (Section 5).

5. Comparative Kinetic Modeling of Enzyme Inactivation

Robust kinetic characterization of enzyme inactivation is central to validating any non-thermal process as pasteurization-equivalent, since regulatory frameworks in most jurisdictions rely on indicator enzyme inactivation (classically, alkaline phosphatase) as a surrogate marker for pathogen inactivation and process adequacy in thermally processed milk. Extending this validation logic to non-thermal technologies requires kinetic models appropriate to each technology's distinct inactivation behavior. Table 2 summarizes the principal kinetic frameworks applied across HPP, PEF, and ultrasound.

Table 2. Kinetic models commonly applied to enzyme and microbial inactivation across non-thermal milk processing technologies.

Model	Governing Assumption	Typical Application in This Context	Key Parameters
First-order (log-linear)	Constant fractional inactivation rate per unit time at fixed intensity	Thermal and HPP inactivation of ALP, LPO, and vegetative microorganisms under isothermal/isobaric conditions	Rate constant k ; D-value (time for 1-log reduction); z -value (temperature/pressure sensitivity)
Weibull (Peleg/Mafart)	Inactivation rate distributed over a population with non-linear (concave/convex) survival curves	Non-linear survival curves frequently observed in HPP and PEF enzyme/microbial inactivation	Shape parameter (n); scale parameter (b or δ , time for first decimal reduction)
Quasi-chemical / biphasic	Two or more sub-populations with distinct sensitivities; sequential or parallel reaction steps	HPP inactivation kinetics showing tailing or shoulder effects	Multiple rate constants; fractional resistant population
PEF-specific empirical models	Field strength and treatment time as primary independent variables	PEF inactivation of enzymes/microorganisms	Critical field strength (E_c); treatment time exponent

	(often combined with a Hulsheger-type or Fermi model)	as a function of E (kV/cm) and t (μs)	
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5.1 First-Order (D/z-Value) Kinetics

Classical first-order kinetics, expressed through decimal reduction time (D-value) and temperature sensitivity (z-value) parameters originally developed by Bigelow, Ball, and Stumbo for thermal processing, remain the most widely applied framework and continue to underpin standard practices for validating pasteurization via alkaline phosphatase and lactoperoxidase inactivation assays [13], [14]. This approach assumes a constant fractional inactivation rate at fixed processing intensity, which holds reasonably well for isothermal thermal treatment and, within limited ranges, for isobaric HPP treatment.

5.2 Weibull and Non-Linear Models

Survival curves for both microorganisms and enzymes frequently deviate from strict log-linearity, showing concave (tailing) or convex (shoulder) behavior that first-order kinetics cannot capture. The Weibull model, in both its Peleg and Mafart parameterizations, has been shown to describe such non-linear survival curves more accurately than the classical log-linear model in numerous thermal and non-thermal contexts [15], [16]. This flexibility is particularly relevant to HPP, where pressure-induced enzyme activation at moderate pressures followed by inactivation at higher pressures produces distinctly non-monotonic behavior poorly suited to single-rate-constant models, and specialized kinetic reviews have compiled HPP-specific models — including quasi-chemical and biphasic formulations — to address these deviations [17].

5.3 PEF-Specific Kinetic Models

Because PEF inactivation depends jointly on electric field strength and treatment time rather than temperature, PEF-specific kinetic models incorporate field strength as an independent variable analogous to temperature in thermal models, often applying a critical field strength threshold (E_c) below which inactivation is negligible. A dedicated review of kinetic models for PEF-based inactivation of microorganisms and enzymes catalogues these field-strength-dependent formulations and highlights their necessity given that conventional D/z-value kinetics, calibrated against temperature, are not directly transferable to a technology whose principal driving variable is electrical rather than thermal [18].

5.4 Cross-Technology Comparison

Taken together, the kinetic literature suggests a consistent pattern: first-order models remain adequate first approximations for many thermal and some HPP and PEF applications, particularly at fixed processing intensity, but genuine process optimization and cross-technology comparison increasingly rely on non-linear (Weibull, biphasic, or technology-specific empirical) models that better capture the non-monotonic, synergistic, or threshold-dependent inactivation behaviors characteristic of HPP, PEF, and ultrasound. This has direct practical implications: process validation protocols developed for one non-thermal technology cannot be assumed to transfer directly to another, and enzyme-specific kinetic parameters must be re-derived for each technology-enzyme-matrix combination rather than extrapolated from thermal precedent.

6. Technology Comparison, Regulatory Status, and Scale-Up Challenges

Table 1 summarizes the operating ranges, mechanisms, and characteristic protein/enzyme effects of the three technologies discussed in this review.

Table 1. Comparative overview of HPP, PEF, and ultrasound for milk preservation.

Technology	Typical Operating Range	Primary Inactivation Mechanism	Effect on Caseins	Effect on Key Whey/Bioactive Proteins
HPP	400–600 MPa, 1–10 min, 4–25 °C	Pressure-induced disruption of non-covalent bonds; casein micelle dissociation	Reduces micelle size; hydrophobic/electrostatic bonds disrupted while covalent bonds retained	β -lactoglobulin denatures above ~400 MPa via free thiol exposure; immunoglobulins and lactoferrin comparatively pressure-stable at moderate levels
PEF	15–40 kV/cm, μ s-scale pulses, near-ambient bulk temperature	Electroporation/dielectric rupture of microbial membranes; localized electrochemical effects on proteins	Minimal micelle-level disruption reported at moderate field strengths	Whey proteins and immunoglobulins largely retained; some indigenous enzymes partially inactivated depending on field strength and treatment time
Ultrasound (power US)	20–100 kHz, high intensity, with or without mild heat (thermosonication)	Acoustic cavitation: microbubble collapse generates localized shear, heat, and radical formation	Can reduce fat globule and casein aggregate size; limited effect on primary structure alone	Whey protein and enzyme denaturation occurs mainly when ultrasound is combined with heat; ultrasound alone has minimal effect on native enzymes

Despite strong laboratory-scale evidence for all three technologies, commercial and regulatory adoption for milk specifically remains limited. HPP is well established for other food categories but is not yet approved for fluid milk pasteurization in several major markets, including the United States, reflecting the incomplete state of research jointly validating microbial safety and bioactive protein preservation under a single set of operating parameters [1]. PEF technology has demonstrated effectiveness for dairy product safety with minimal quality impact primarily at laboratory and pilot scale, and extensive research at commercial scale remains necessary before widespread industrial implementation [3]. Ultrasound faces a related challenge: because meaningful enzyme inactivation typically requires the addition of heat, purely non-thermal ultrasound applications for full pasteurization-equivalent processing remain constrained, positioning thermosonication as a hybrid rather than fully non-thermal solution [12].

7. Future Directions

Several research priorities emerge from this comparative analysis:

- Joint validation studies that simultaneously assess microbial log-reduction and bioactive protein/enzyme preservation under identical processing conditions, rather than treating these as separate research questions.
- Development and cross-validation of technology-specific kinetic models (Weibull, biphasic, PEF field-strength models) against standard first-order benchmarks, to establish which framework is most appropriate for each enzyme-technology-matrix combination.

- Systematic investigation of combined/hurdle approaches (e.g., moderate HPP with mild heat, PEF with preheating, thermosonication) that may achieve synergistic microbial inactivation while limiting bioactive protein loss more effectively than any single technology alone.
- Pilot- and commercial-scale process validation studies, particularly for PEF and ultrasound, to close the gap between laboratory-demonstrated potential and industrially deployable, regulator-accepted processes.
- Standardized reporting of processing parameters (pressure/time/temperature for HPP; field strength/pulse width/specific energy for PEF; frequency/intensity/temperature profile for ultrasound) to enable meaningful cross-study kinetic comparison.

8. Conclusion

High-pressure processing, pulsed electric field processing, and power ultrasound each offer genuine advantages over thermal pasteurization for preserving heat-labile bioactive milk proteins, but none is a universal drop-in replacement. HPP achieves strong microbial inactivation at pressures that can simultaneously compromise pressure-sensitive whey proteins such as β -lactoglobulin and immunoglobulin G, while better preserving lactoferrin and alkaline phosphatase structure. PEF offers the least thermal impact and the strongest protein-preservation profile among the three, but enzyme inactivation is more limited and highly dependent on field strength and specific energy input. Ultrasound alone has minimal effect on indigenous milk enzymes and typically requires combination with mild heat to achieve meaningful inactivation, positioning thermosonication as a hybrid rather than purely non-thermal approach. Across all three technologies, enzyme inactivation kinetics frequently deviate from classical first-order behavior, and accurate process validation increasingly depends on Weibull, biphasic, or technology-specific kinetic models rather than thermal-processing precedent. Continued joint investigation of microbial safety and bioactive protein preservation, supported by robust and technology-appropriate kinetic modeling, will be essential to moving these green processing technologies from laboratory demonstration to validated commercial practice in dairy science.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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