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IMPACT OF PASTEURIZATION ON THE INTEGRITY OF BIOACTIVE COMPONENTS IN DONOR HUMAN MILK: A COMPREHENSIVE LITERATURE REVIEW

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Abstract **Background:** Human milk is the gold standard for neonatal nutrition, particularly for preterm and very low birth weight (VLBW) infants. When mother's own milk is unavailable or insufficient, donor human milk provided through human milk banks is the preferred alternative. To ensure microbiological safety, donor human milk undergoes pasteurization; however, thermal processing may compromise bioactive components essential for immune protection and gastrointestinal development.
Objective: This review aimed to evaluate current evidence regarding the effects of pasteurization on the preservation of bioactive components in donor human milk and to assess emerging processing technologies and their clinical implications.
Methods: A structured narrative review was conducted using evidence from randomized controlled trials (RCTs), systematic reviews, scoping reviews, and international clinical guidelines published primarily between 2015 and 2026. Literature was identified through PubMed/MEDLINE, Scopus, Cochrane Library, and ScienceDirect.
Results: Holder Pasteurization (HoP) (62.5°C for 30 minutes) effectively inactivates microbial pathogens but reduces several key immunological components, including secretory immunoglobulin A (17.6–50%), lactoferrin (5.6–80%), and lysozyme (9.8–35%), while nearly eliminating bile salt–stimulated lipase activity. In contrast, human milk oligosaccharides (HMOs), most minerals, and water-soluble vitamins remain relatively stable. Alternative technologies, including High-Pressure Processing (HPP) and High-Temperature Short-Time (HTST) pasteurization, demonstrated improved preservation of bioactive activity.
Conclusion: Despite reducing selected bioactive components, pasteurized donor human milk remains clinically superior to infant formula and contributes to lowering the risk of necrotizing enterocolitis (NEC). Further development of advanced processing technologies and optimized thermal control is needed to maximize bioactive preservation.

Keywords: donor human milk; Holder pasteurization; bioactive components; high-pressure processing

BACKGROUND

Human milk is not merely a source of macronutrients but a dynamic biological fluid containing living immune cells, immunoglobulins, cytokines, enzymes, hormones, growth factors, and a functional microbiome that adaptively responds to the physiological needs of the infant.[1] These bioactive components provide direct protection against infection and facilitate gastrointestinal and immune system maturation, which is particularly important in preterm infants with immature intestinal mucosal barriers.[2]

When mother's own milk (MOM) is insufficient or unavailable, current international clinical guidelines including recent recommendations from the World Health Organization (WHO) for the care of preterm and low birth weight infants recommend pasteurized donor human milk from human milk banks as the preferred enteral alternative.[3,4] Donor human milk has consistently been associated with reduced neonatal morbidity, particularly necrotizing enterocolitis (NEC), compared with formula feeding.[3,4]

To prevent transmission of infectious agents, including viral pathogens such as human immunodeficiency virus (HIV) and cytomegalovirus (CMV), as well as bacterial contamination, human milk banks worldwide implement strict microbiological safety standards.[5] Holder Pasteurization (HoP) remains the standard processing method internationally and is recommended by most human milk banking guidelines.[5] This process involves heating donor human milk at 62.5°C for 30 minutes, a protocol designed to ensure pathogen inactivation while maintaining nutritional quality more effectively than conventional high-temperature sterilization methods.[6] However, despite its proven microbiological efficacy, thermal processing may induce protein denaturation and structural modifications in heat-sensitive bioactive fractions, resulting in partial loss of the immunological and functional properties of donor human milk.[7]

This issue has become increasingly important as emerging evidence suggests that reduced bioactivity following milk processing may contribute to slower growth among recipients of donor human milk compared with infants receiving mother's own milk.[8] In addition, substantial methodological heterogeneity among studies—particularly differences in pre-analytical sample handling and mixing techniques—has led to inconsistent findings regarding post-pasteurization nutrient and fat measurements.[9] These limitations emphasize the need for a more rigorous and contemporary synthesis of current evidence.

Therefore, this review aims to: (1) critically evaluate current evidence regarding the extent of degradation of bioactive components in donor human milk following pasteurization; (2) synthesize quantitative findings from primary studies and systematic reviews into a comparative framework; and (3) assess the future potential of alternative processing technologies and their implications for clinical practice and policy development in human milk banking, particularly in developing countries.

METHODS

This literature review was conducted using a structured narrative review approach to synthesize evidence from primary research articles, clinical trials, systematic reviews, scoping reviews, and official guidelines related to human milk banking and the biochemical changes occurring after donor human milk pasteurization. A literature search was performed across electronic databases, including PubMed/MEDLINE, Scopus, Cochrane Library, and ScienceDirect. The search strategy combined the following keywords: "donor human milk" OR "donor milk" OR "human milk bank" AND "Holder pasteurization" OR "high-pressure

processing" OR "high-temperature short-time" OR "bioactive components" OR "immunoglobulin A" OR "lactoferrin" OR "lysozyme". Only articles published in English and appearing in peer-reviewed journals were considered for inclusion.

Inclusion and Exclusion Criteria

The following eligibility criteria were applied:

1. Primary studies, including randomized controlled trials (RCTs), cohort studies, and matched-pair comparative studies, comparing raw donor human milk with milk processed using thermal and non-thermal technologies.
2. Systematic reviews, scoping reviews, and meta-analyses evaluating the impact of donor human milk processing on nutritional composition and bioactive activity.
3. Official clinical guidelines issued by internationally recognized human milk banking organizations, including HMBANA, the Canadian Paediatric Society, WHO, and ESPGHAN.
4. Publications published primarily within the last ten years (2015–2026) to ensure contemporary relevance, while selected landmark studies were retained because of their methodological importance.
5. Duplicate publications, opinion papers lacking primary data, conference abstracts without full manuscripts, and animal studies without direct translational relevance were excluded from the primary quantitative synthesis, although selected studies were considered as contextual mechanistic evidence.

Data extraction focused on quantitative indicators, including percentage changes in concentration and/or activity of secretory immunoglobulin A (sIgA), lactoferrin, lysozyme, human milk oligosaccharides (HMOs), macronutrients, vitamins, minerals, and clinical outcomes associated with pasteurized donor human milk administration, including incidence of necrotizing enterocolitis (NEC), enteral feeding tolerance, and neonatal intensive care unit (NICU) length of stay. The extracted findings were synthesized narratively and presented in comparative tables to facilitate cross-study interpretation and identification of consistent trends.

RESULTS AND DISCUSSION

The literature synthesis demonstrated a consistent pattern indicating that the effects of pasteurization on donor human milk are component-selective rather than universally degradative. Certain heat-sensitive bioactive constituents undergo substantial reduction following thermal processing, whereas other nutritional and functional components remain relatively stable. Table 1 summarizes the quantitative changes observed in major bioactive and nutritional components of donor human milk following Holder Pasteurization (HoP).

Table 1. Summary of the Effects of Holder Pasteurization on Bioactive and Nutritional Components of Donor Human Milk

Bioactive Component	Post-HoP Change	Reduction Range (%)
Secretory IgA (sIgA)	Significant decrease	17.6–50
Lactoferrin	Marked decrease	5.6–80
Lysozyme	Variable reduction	9.8–35
Human Milk Oligosaccharides (HMOs)	Relatively stable	Minimal / not significant
Macronutrients (fat, protein, carbohydrate)	Generally preserved; fat measurements varied methodologically	−28.6 to +19.4
Vitamins and minerals (thiamine, calcium, copper)	Minimal loss	<15
Bile salt–stimulated lipase (BSSL) activity	Nearly complete inactivation	~100

Three major patterns emerged from the evidence synthesis.

First, heat-labile immunological proteins—including secretory immunoglobulin A (sIgA), lactoferrin, and lysozyme—showed the most consistent reductions following Holder Pasteurization. Reported losses varied substantially across studies, ranging from 5.6% to more than 80% for lactoferrin, suggesting that differences in pasteurization equipment, thermal profiles, and analytical methodologies contribute significantly to observed variability.

Second, non-protein bioactive compounds, particularly human milk oligosaccharides (HMOs), as well as most micronutrients (including calcium, copper, and thiamine) and energy-related macronutrients, demonstrated considerable thermal stability under HoP conditions (62.5°C for 30 minutes), with most reported losses remaining below 15%. Third, enzymatic activity appeared to be especially vulnerable to thermal exposure. Bile salt–stimulated lipase (BSSL), a key enzyme involved in neonatal fat digestion, was consistently reported to undergo almost complete inactivation following thermal processing. This finding was observed across studies evaluating both HoP and alternative processing approaches.

Comparative evaluation of processing technologies further demonstrated that emerging non-thermal and semi-thermal methods—including High-Pressure Processing (HPP) and High-Temperature Short-Time (HTST) pasteurization—consistently achieved superior preservation of bioactive compounds compared with conventional HoP. Studies comparing HTST with standard HoP reported significantly higher post-processing retention of immunological markers, including IgA, IgG, IgM, and leptin. Similarly, HPP demonstrated greater preservation of nutritional and functional bioactive components under optimized pressure conditions.

From a clinical perspective, evidence synthesized from systematic reviews and large cohort studies consistently indicated that pasteurized donor human milk, despite reduced bioactivity, remained associated with clinically meaningful benefits compared with preterm infant formula, particularly through reduced incidence of necrotizing enterocolitis (NEC) and improved enteral feeding tolerance.

This review demonstrates that the impact of pasteurization on donor human milk is selective and largely determined by the physicochemical characteristics of individual bioactive components rather than representing universal nutritional degradation. While Holder Pasteurization (HoP) remains the internationally accepted standard for ensuring microbiological safety in human milk banking, accumulated evidence indicates a measurable trade-off between pathogen inactivation and preservation of biological functionality.[6,7]

Among the evaluated bioactive compounds, immunologically active proteins appear to be the most vulnerable to thermal exposure. In addition to secretory immunoglobulin A (sIgA), lactoferrin—a multifunctional iron-binding glycoprotein with potent bacteriostatic activity—and lysozyme, an antibacterial enzyme responsible for bacterial cell wall lysis, consistently demonstrated substantial reductions after HoP. Reported losses ranged from approximately 57–65% in earlier studies and exceeded 80% in more recent multicenter analyses using more sensitive analytical approaches.[6,9,20] This broad variability suggests that protein degradation is influenced not only by nominal processing temperature but also by additional factors, including temperature transition time (come-up time), sample volume, and equipment calibration.[10]

Importantly, evidence from recent process-optimization studies suggests that a considerable proportion of this variability may be modifiable. Buffin et al. demonstrated that highly controlled HoP systems with precise temperature gradients and exposure duration substantially improved retention of bioactive proteins, limiting reductions to 17.6% for IgA, 5.6% for lactoferrin, and 9.8% for lysozyme.[10] These findings support the concept that improvements in process engineering may significantly enhance bioactive preservation without requiring complete replacement of conventional HoP technology.

Not all biologically active components are adversely affected by thermal processing. Human Milk Oligosaccharides (HMOs), which function as prebiotics and pathogen decoys, appear to remain highly stable following Holder Pasteurization.[9] Recent large-scale multicenter evaluations further confirmed that HoP exerts minimal to negligible effects on total energy content, essential nutrients, HMOs, B vitamins, and mineral concentrations in donor human milk.[20]

Similarly, comparative studies evaluating pooled donor milk samples demonstrated that overall energy value, carbohydrate content, and most lipid fractions remain quantitatively preserved after HoP treatment.[11] However, a recent scoping review identified substantial inconsistency in reported post-pasteurization fat concentrations, ranging from −28.6% to +19.4%, and concluded that much of this variation originated from differences in pre-analytical sample mixing and handling procedures rather than thermal effects alone.[18] These findings emphasize the importance of standardizing sampling and analytical protocols to improve cross-study comparability in future donor human milk research.

From a digestive physiology perspective, evidence synthesized from in vitro and in vivo studies indicates that pasteurization consistently reduces lipolysis while increasing proteolytic modification of lactoferrin and casein, although total protein hydrolysis appears relatively preserved.[20] Reduced post-pasteurization lipid digestion may partially explain the slower growth trajectories observed among preterm infants receiving donor human milk compared with those receiving mother's own milk (MOM), although further translational research is required.[19]

One of the most consistent findings across the literature is the near-complete inactivation of bile salt–stimulated lipase (BSSL) following both HoP and HTST processing. Comparative studies demonstrated that although HTST preserved selected nutritional parameters more

effectively than conventional HoP, BSSL activity remained highly susceptible to thermal exposure.[16] Considering the critical role of BSSL in long-chain triglyceride digestion among preterm infants with immature pancreatic lipase secretion, preservation of enzymatic activity should remain a major target for future processing innovations.

Consequently, recent pediatric and nutrition research has increasingly focused on alternative processing technologies. High-Pressure Processing (HPP) has emerged as one of the most promising non-thermal approaches. Experimental studies demonstrated that HPP provides superior preservation of nutrient composition, lactoferrin, lysozyme, and lipase activity compared with conventional HoP, particularly under pressure conditions below 600 MPa without heat combination.[12,13] Additional animal studies also reported improved growth outcomes and superior antioxidant potential following administration of HPP-treated donor milk, suggesting functional advantages beyond compositional preservation.[22,23]

Another emerging strategy is High-Temperature Short-Time (HTST) pasteurization, which applies higher temperatures for substantially shorter durations. Evidence suggests improved preservation of selected nutritional and protective compounds compared with conventional HoP.[15,18] Recent systematic evaluations concluded that although HoP remains the global standard because of its established safety profile, HTST and HPP currently represent the most mature alternative technologies for future implementation in donor human milk banking.[21]

Despite unavoidable reductions in selected bioactive components, pasteurized donor human milk continues to provide clear clinical advantages over preterm infant formula. Unlike formula, donor human milk retains unique biological elements, including immunological activity, HMOs, and endogenous growth-related factors.[9] Clinical evidence, including systematic reviews and meta-analyses, consistently demonstrates that donor human milk reduces the risk of necrotizing enterocolitis (NEC), improves enteral feeding tolerance, and may shorten neonatal intensive care unit (NICU) length of stay among preterm and vulnerable infants.[4,14]

Therefore, while maximizing bioactive preservation remains an important long-term objective, current evidence strongly supports pasteurized donor human milk as the preferred second-line enteral nutrition strategy when mother's own milk is unavailable.

CONCLUSION

Pasteurization, particularly the conventional Holder Pasteurization (HoP) method, represents a critical balance between ensuring microbiological safety and preserving the functional bioactivity of donor human milk. Evidence synthesized in this review demonstrates that the effects of pasteurization are component-specific. Thermal processing substantially reduces selected immunological proteins and enzymatic activity, while most human milk oligosaccharides (HMOs), macronutrients, and micronutrients remain relatively preserved.

The variability observed across studies suggests that differences in thermal precision and pre-analytical sample handling contribute significantly to post-processing outcomes. These findings indicate that optimization of processing conditions and operational standardization may substantially improve bioactive retention without requiring complete replacement of established HoP systems.

Future directions should prioritize integration of more precise thermal control strategies, gradual implementation of semi-thermal approaches such as High-Temperature Short-Time (HTST) pasteurization, and continued development and standardization of non-thermal technologies including High-Pressure Processing (HPP). Additional studies with standardized

methodologies, larger sample sizes, and long-term clinical outcome evaluation are necessary to strengthen the translational evidence supporting donor human milk processing.

Overall, despite unavoidable reductions in selected bioactive components, pasteurized donor human milk remains the preferred alternative when mother's own milk is unavailable and continues to provide clinically meaningful benefits for preterm and vulnerable infants compared with infant formula.

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