



Thermal Stability of Human Milk Bioactives: A Global Bibliometric Overview

Dilma'aarij Agustia^{1*}, Bilqis Nur Mustofa¹

¹Department of Health, Universitas Satya Terra Bhinneka, Medan City, North Sumatra, 20128, Indonesia

*Corresponding author: dilmaagustia@satyaterrabhinneka.ac.id

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HIGHLIGHTS

- ❖ The most extensively studied research themes include pasteurization, food safety, and microbiological quality

ABSTRACT

Human milk is the optimal source of nutrition for infants, providing essential nutrients and bioactive components crucial for growth and development. However, these components are sensitive to post-expression handling, particularly thermal processes like storage, pasteurization, and heating. This study maps the global research landscape on the thermal stability of human milk bioactive components and identifies trends, key contributors, and research gaps. A bibliometric analysis was conducted using the Scopus database for publications from 2000 to 2023. The findings reveal a significant upward trend in publications over the last decade, with research predominantly led by the United States and China through strong international collaborations. While themes such as pasteurization and microbiological quality are well established, research on the thermal stability of non-protein bioactive components, inter-component interactions, and their long-term clinical impacts on infant health remains scarce. These results provide a foundation for developing evidence-based guidelines for human milk handling and highlight priority areas for future investigation.



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INTRODUCTION

Human milk is the optimal source of nutrition for infants because it provides essential macro- and micronutrients and a wide range of bioactive components that support infant growth, development, and immune function. Bioactive components of human milk, such as immunoglobulins, enzymes, hormones, cytokines, and growth factors, provide protective and regulatory functions that infant formula cannot fully replicate (Carr et al., 2021). The bioactive components of human milk are dynamic and may undergo changes in both concentration and quality. Several factors influence these changes, including biological and environmental factors, as well as post-expression handling practices. Understanding these factors is essential to ensure that infants continue to receive the optimal benefits of human milk (Peila et al., 2016). The bioactive components of breast milk that are susceptible to temperature changes, namely lactoferrin, immunoglobulins, lysozyme, and growth factors, undergo structural changes due to the increase in temperature caused by kinetic energy, leading to protein aggregation and a subsequent decrease in the biological activity of breast milk.

Accordingly, maintaining the quality and biological functionality of human milk is a key priority in maternal and child health practice (García-Lara et al., 2012). In public health, maintaining the quality of expressed human milk is crucial to ensure infants continue to receive the optimal benefits of its bioactive components. Therefore, understanding the thermal stability of human milk bioactive components provides a foundation for developing safe and effective guidelines for human milk storage and heating. This has driven a growing body of research examining the thermal stability of human milk from multiple perspectives, including biochemistry, food technology, and maternal and child health (Chang et al., 2013).

Although the number of studies continues to increase, to date there have been limited efforts to specifically map the global development of research on the thermal stability of bioactive components in human milk. While previous bibliometric studies have explored broader themes, such as general human milk composition or the processing techniques in bovine dairy products, none have explicitly integrated and synthesized the global research landscape focused exclusively on the intersection of thermal processing and the preservation of human milk's unique bioactives. Without a clear map, it is difficult to identify the countries and institutions that contribute most significantly, the research themes most extensively investigated, and the areas that remain underexplored. Bibliometric analysis can address this gap by providing a comprehensive overview of research dynamics within a particular field (Salcedo et al., 2018). Through bibliometric analysis, this article aims to contribute scientifically by mapping global research trends, identifying key research topics, and proposing directions for future studies related to the thermal stability of human milk bioactive components. This information is expected to serve as a valuable reference for researchers, healthcare practitioners, and policymakers in the development of evidence-based research and human milk management practices.

MATERIALS AND METHODS

Methods

This study applied a bibliometric approach to map the scientific literature on the thermal stability of bioactive components related to human milk indexed in the Scopus database. Primary data were extracted on January 1, 2026, from the Scopus database. The search was limited to the Title, Abstract, and Keywords (TITLE-ABS-KEY) fields to ensure relevance. The search was conducted using Boolean techniques with the keywords TS = ("human milk" OR "breast milk") AND TS = (bacteria) AND TS = (quality) AND TS = ("thermal stability" OR heating OR pasteurization) AND TS = (bioactive compounds). From an initial search of 2000 documents, 1025 publications met the inclusion criteria after screening.

Initially, Scopus's automated filters removed non-English publications and non-article document categories. To ensure they addressed the intersection of human milk, bioactives, and thermal stability, the remaining titles and abstracts were manually screened in the second stage. Excluded were documents that were deemed unrelated to the main study topic. After this two-stage screening procedure, 1025 publications that met the inclusion criteria were retained for bibliometric analysis. **Figure 1** illustrates the selection process in detail using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework. Data analysis was comprehensively performed using VOSviewer (version 1.6.17) and the web-based application Biblioshiny (RStudio).

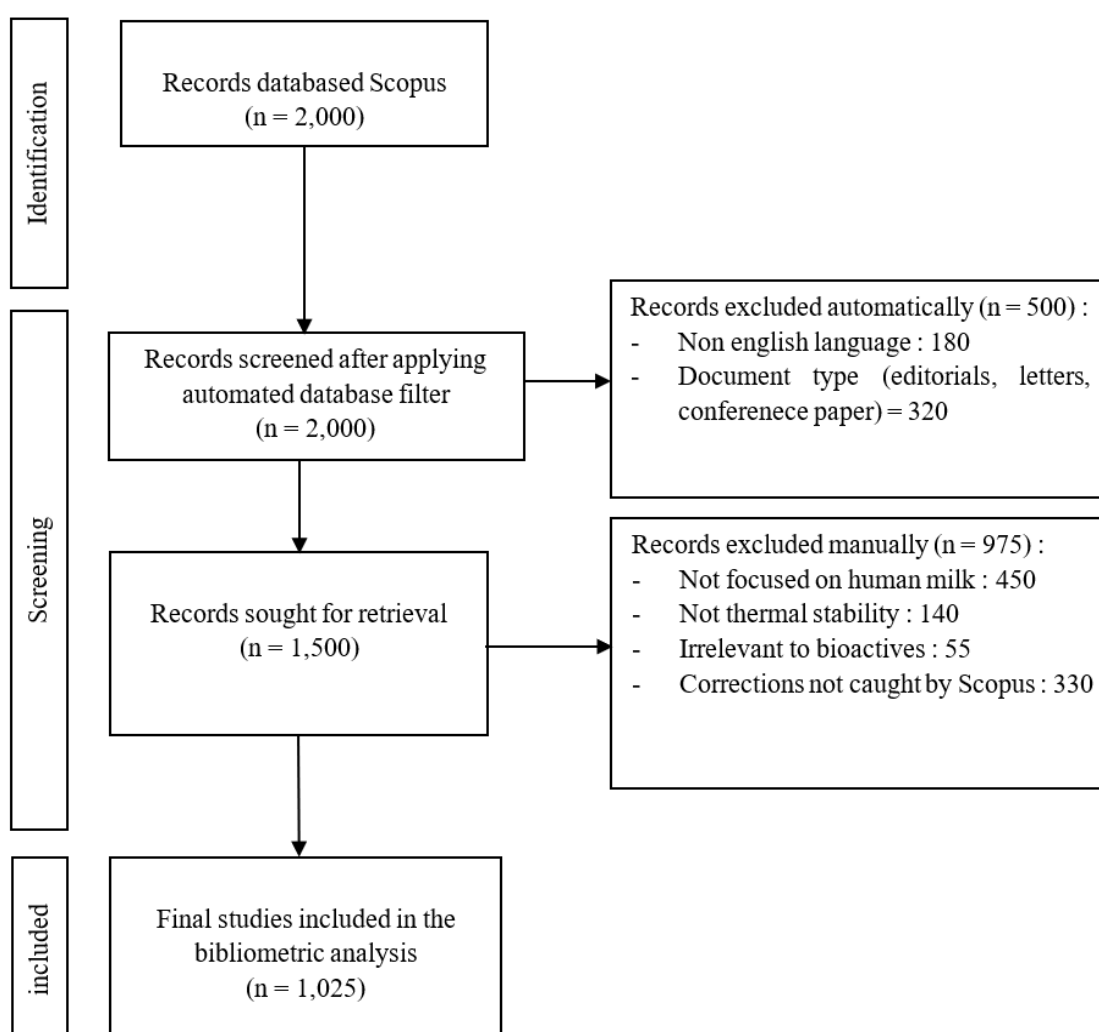


Figure 1. PRISMA flow diagram outlining the search strategy for the review

Inclusion and exclusion criteria

The study period was defined as 2000–2023, from when this topic was first published globally through the most recent period, to obtain a comprehensive understanding based on titles, abstracts, and keywords. The study was limited to English-language publications, with document types restricted to original research articles, review articles, and early access articles. Exclusion criteria were applied to documents such as conference proceedings, letters, corrections, and editorial materials to ensure the quality and reliability of the analyzed data.

Statistical analysis and bibliometric indicators

Bibliometric analysis was employed to visualize quantitative relationships among scientific literature. Using VOSviewer software developed by Leiden University, bibliometric networks were constructed to map interconnections among authors, institutions, documents, and keywords. A minimum occurrence criterion of five for keywords was used, and a minimum citation threshold of twenty citations for author co-citation analysis to set the network construction parameters in VOSviewer. The Association Strength approach was used to normalize the network. The VOSviewer clustering algorithm was used for keyword and author clustering, with a resolution parameter of 1.00. Each component is shown as a node (point or circle) in the resulting network maps, with the size of the circle indicating the quantity or frequency of publications. Centrality indices determine an element's significance or impact in the knowledge map; nodes with high centrality serve as important links in the research network.

In addition, annual publication growth trends were analyzed using Microsoft Excel 2020. To qualitatively evaluate changes in research productivity over time, this required tracking annual total publication volume and dividing the analysis into discrete historical eras. In the resulting network maps, each element is represented as a “node” (point/circle), with the circle size reflecting the volume or frequency of publications. The level of influence or significance of an element within the knowledge map is determined by centrality indices, whereby nodes with high centrality function as key connectors within the research network.

To visualize collaboration networks, analysis was done at the national and author levels. A particular author or nation is represented by a node on these maps. When two nodes are linked together, it means they are co-authors and have co-published a document. The link's thickness, which indicates how frequently they work together, represents the link's overall strength. To find research schools and intellectual structures, author co-citation analysis was done. Nodes in this analysis represent referenced authors. Two writers who are regularly quoted together in a third publication's reference list are connected by a link. Research themes or theoretical underpinnings are thought to be comparable among authors who feature in the same cluster.

The thematic landscape and progression were mapped using keyword co-occurrence analysis. Keywords taken from titles, abstracts, and author keywords are represented as nodes. When two keywords appear together in the same document, it is indicated as a link. Different study themes were identified using cluster colors. An overlay visualization displayed keywords by average publication year to show temporal trends.

RESULTS

Annual number of publications

Figure 2 shows the number of publications on the thermal stability of bioactive components in human milk. In the early 2000s, publication output in this area was very limited, indicating that the topic had not yet become a major focus of global research. In contrast, from 2005 to 2018, the number of studies increased sharply and consistently, suggesting that this research area had emerged as an important global issue. Publication output peaked during 2019–2023. Although the most recent year shows a slight decline, this does not necessarily indicate decreased research interest; rather, it reflects incomplete data for the ongoing year or delays in journal indexing.

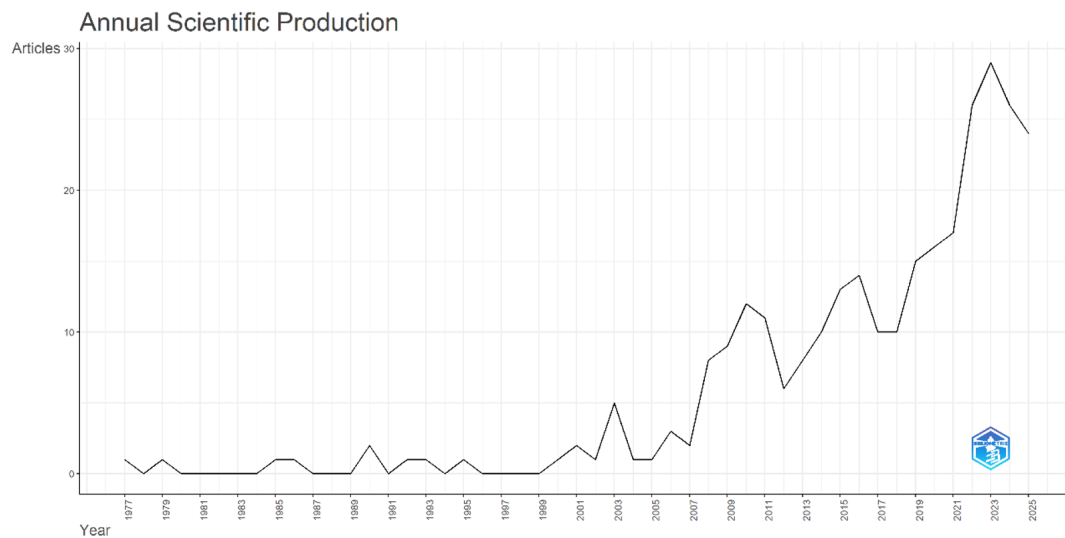


Figure 2. Annual publication trends in research on the thermal stability of bioactive components in human milk, 2000–2023

Co-authorship network

The results indicate that several key authors have been highly productive in advancing this research field, including Nero Luis Augusto, Chen Rong, Ding Juan, Zhang Fan, and Alessandri Giulia. These authors have made significant contributions to research on the thermal stability of human milk content and bioactive components. In addition, **Figure 3** illustrates the author collaboration network in publications related to this field. Several distinct collaboration clusters are observed, indicating that research groups in this area remain largely regionally concentrated.

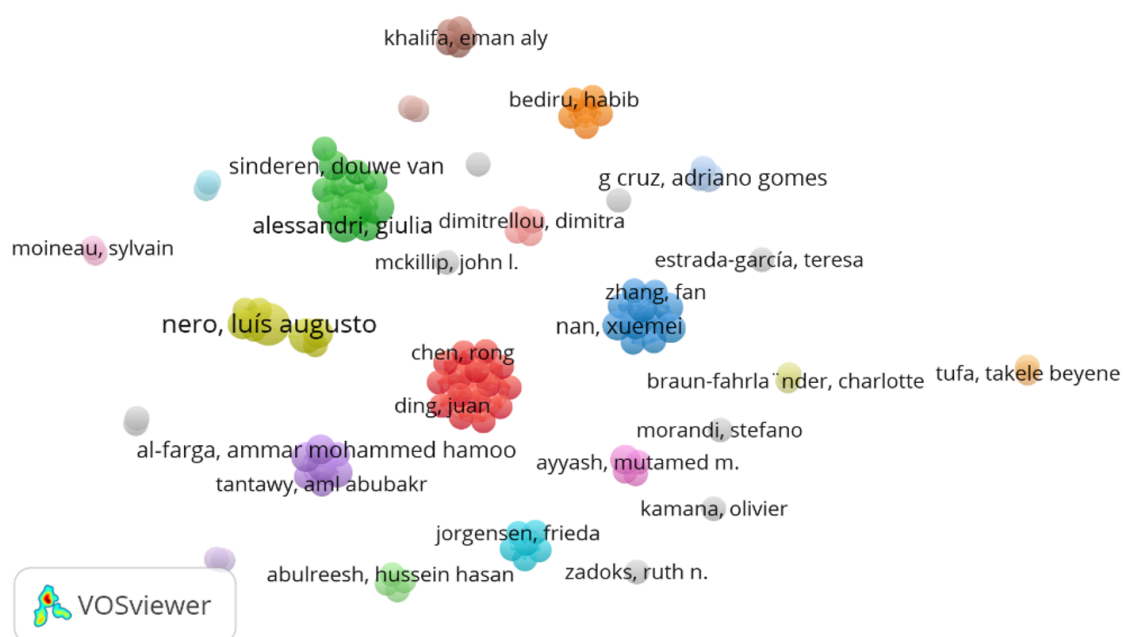


Figure 3. Author co-authorship network on thermal stability of human milk bioactive components

Average number of citations received per article

This figure illustrates the scientific quality and influence of publications in the field under investigation. From 1996 to 2003, citation counts increased gradually, indicating growing scholarly attention

to the stability of bioactive components in human milk. In contrast, from 2004 to 2006, a sharp surge in citations occurred, reaching a peak likely driven by the publication of key seminal articles that established this field as highly significant and laid the foundation for subsequent research. In more recent years, particularly from 2021 to 2024, the average number of citations declined. This trend may be attributed to the relatively recent publication of these articles, as they have not yet had sufficient time to accumulate citations.

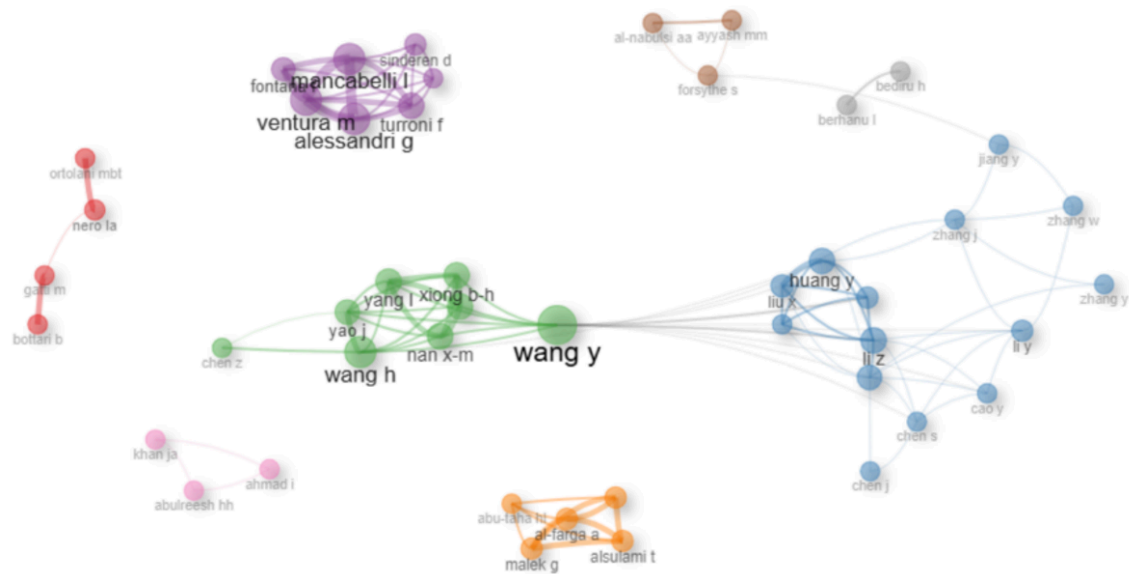


Figure 5. Co-citation frequency of authors

Country-level collaboration network

The global collaboration map illustrates the wide geographical distribution of research on the thermal stability of bioactive components in human milk. China and the United States emerge as the leading contributors, as reflected by their high publication intensity and extensive international collaboration networks. Research activity is primarily concentrated in North America, Europe, and Asia, highlighting strong intercontinental scientific collaboration in this field, as shown in Figure 7.

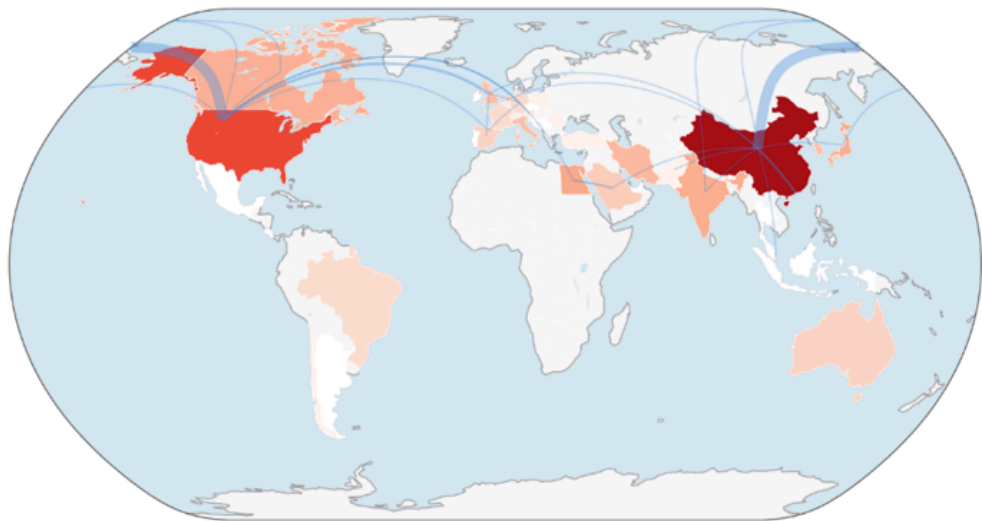


Figure 7. Country-level collaboration network

Article volume analysis

The results indicate that keywords closely related to thermal stability, human milk safety, and milk/human milk processing include raw milk, human milk, food safety, microbiological quality, pasteurization, and infant formula. These findings show that this research topic adopts a multidisciplinary approach, encompassing nutrition, microbiology, midwifery, and public health, as shown in **Figure 8**.

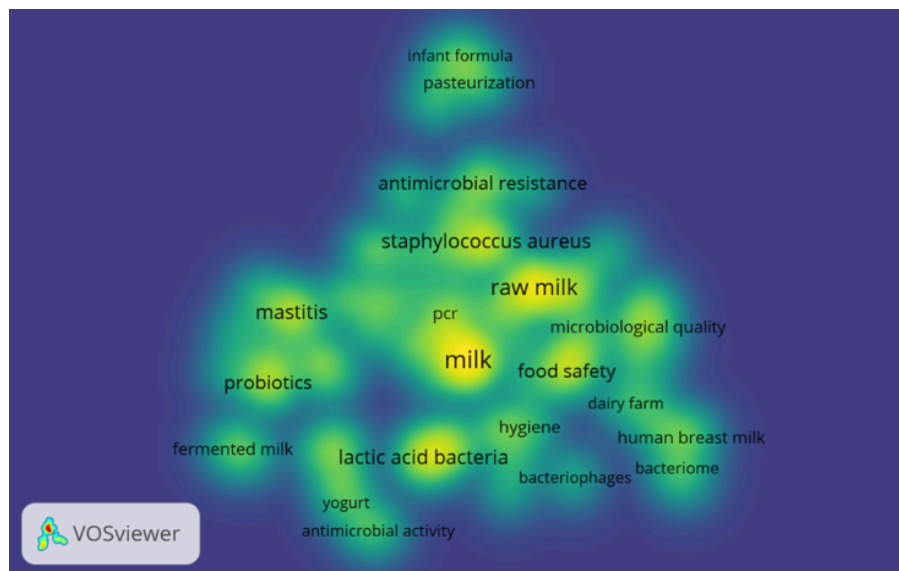


Figure 8. Keyword density visualization

Keyword clustering

The keyword co-occurrence network map shows that the term “milk” is the most dominant keyword and serves as a central hub connecting various research themes, as shown in **Figure 9**. Several major clusters were identified, including thermal processing and pasteurization, microbiological safety, bioactive components and probiotics, as well as antimicrobial resistance. This structure confirms that research on the thermal stability of bioactive components in human milk is inherently multidisciplinary, integrating aspects of food safety, microbiology, and nutritional science.

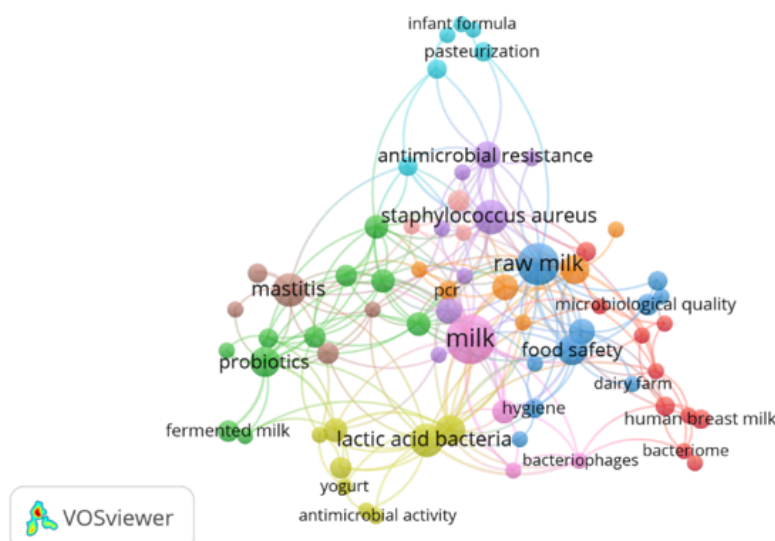


Figure 9. Keyword co-occurrence network visualization

Evolution of research themes and trends

The overlay visualization shown in **Figure 10** reveals a clear temporal evolution of research themes. Early studies primarily focused on general milk microbiology and fermented products, whereas more recent publications increasingly emphasize human milk, pasteurization, and microbiome-related topics. The emergence of keywords such as bacteriome, bacteriophages, and infant formula indicates a growing research interest in the thermal stability and safety of bioactive components in human milk.

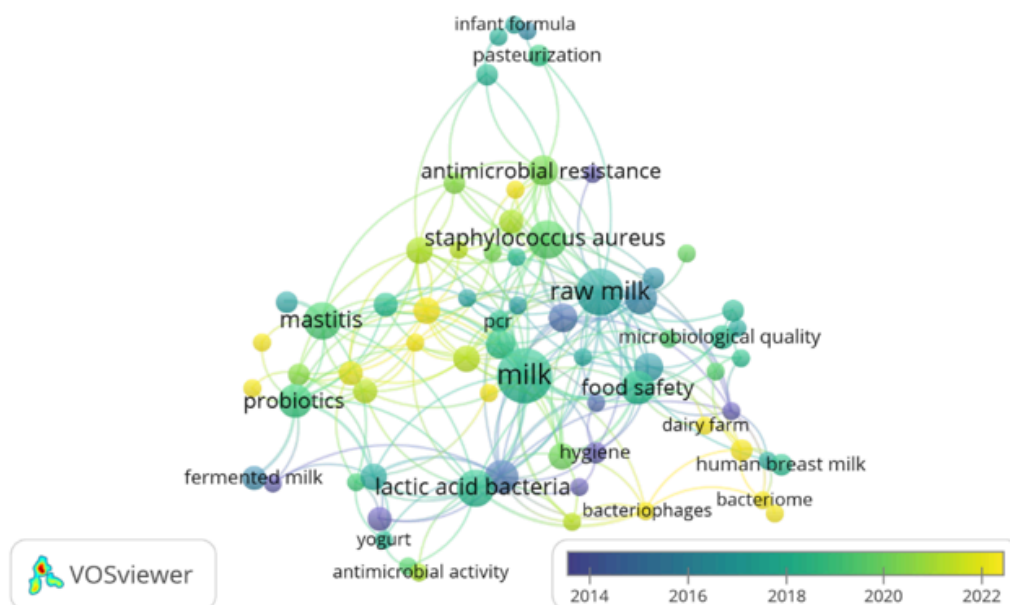


Figure 10. Keyword overlay visualization

DISCUSSION

Thermal stability, human milk, and bioactive components

The bibliometric analysis indicates that the thermal stability of bioactive components in human milk has become a research theme of increasing global interest, particularly over the past two decades. A significant rise in publications and citations reflects growing scientific awareness of the importance of preserving the bioactive properties of human milk during storage and heating processes (Salcedo et al., 2018). The findings of this study are consistent with experimental evidence demonstrating that various bioactive components of human milk, such as immunoglobulins, enzymes, cytokines, and growth factors, are sensitive to heat exposure and may experience a reduction in biological activity when subjected to inappropriate temperature or thermal conditions (Zhang et al., 2023).

The dominance of keywords such as pasteurization, food safety, and microbiological quality indicates that research on the thermal stability of human milk extends beyond nutrition alone, emphasizing the balance between microbiological safety and the preservation of bioactive components. This highlights a major challenge in both field and clinical practice, particularly in the management of expressed human milk and donor human milk. A key contribution of research in this area is the strengthened understanding that thermal treatment may reduce certain bioactive activities in human milk, although it remains necessary to ensure its safety (Eramo et al., 2025).

The analysis reveals several limitations, as most studies still employ laboratory-scale experimental designs with wide variations in heating methods, exposure durations, and measurement parameters that have not yet been standardized. These conditions may affect the consistency of results and hinder

cross-study comparisons. Moreover, most studies focus on specific bioactive components, while the cumulative effects on the overall biological function of human milk have not yet been fully elucidated (Carr et al., 2021).

Research opportunities on the thermal stability and bioactive components of human milk

The bibliometric mapping in this study indicates significant research opportunities in the thermal stability of bioactive components in human milk. While topics related to pasteurization and microbiological safety have been extensively investigated, research on the thermal stability of non-protein bioactive components, interactions among bioactive compounds, and their long-term impacts on infant health remains relatively limited and underexplored (Núñez-Delgado et al., 2024). In addition, the emergence of new keywords such as microbiome, bacteriome, and bacteriophages indicates a shift in research focus toward more integrative and multidisciplinary approaches. Another limitation is the limited number of studies from developing countries, despite the fact that practices related to the storage and heating of expressed human milk are strongly influenced by social, cultural, and household resource contexts. This gap highlights opportunities for context-specific research that can support the development of more comprehensive evidence-based guidelines. Future studies should also explore alternative methods of human milk processing that preserve the stability of bioactive components without compromising microbiological safety. Overall, the findings of this review contribute to mapping the global research landscape on the thermal stability of human milk and provide a scientific foundation for clinical practices, policies for the management of expressed and donor human milk, and more focused, application-oriented directions for future research.

CONCLUSION

This bibliometric analysis summarizes two decades of worldwide research, showing that although studies on the thermal stability of human milk are growing quickly, the main focus has remained on balancing microbiological safety with the preservation of bioactive proteins. This synthesis shows a substantial translational gap between laboratory-scale experimental data and the pressing need for standardized, evidence-based methods in clinical and operational contexts, going beyond recurring findings. As a result, this study has significant practical ramifications for the prompt creation of strong management practices in Human Milk Banks (HMB) and Neonatal Intensive Care Units (NICU). To ensure that hospitalized and premature newborns receive the best possible bioactive nutrition, it is imperative to transfer the mapping evidence into standardized donor human milk processing processes and bedside expressed milk management standards. Future studies must shift from isolated protein analysis to integrative methods to close the highlighted research gaps. Priority should be given to long-term clinical trials monitoring the real health outcomes in infants consuming thermally processed milk, mechanistic studies revealing the thermal stability of non-protein components, lipids, and their inter-component interactions, and context-specific research in developing nations to modify handling guidelines to local resources and sociocultural practices, in addition to investigating non-thermal technologies like High Pressure Processing (HPP) combined with multi-omics. Establishing a thorough worldwide basis for human milk management that optimizes newborn health depends on these initiatives.

AUTHOR CONTRIBUTIONS

DRA: Conceptualization, Methodology, Software/Validation, Formal Analysis, Investigation, Data Curation, Writing- Original Draft, Writing- Review, Writing- Editing, and Funding Acquisition. **BNM:** Conceptualization, Software/Validation, Formal Analysis, Investigation, Data Curation, Writing-Original Draft, Writing-Editing,

Project Administration, and Funding Acquisition.

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COMPETING INTERESTS

The authors declare that there are no competing interests related to the publication of this article.

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ADDITIONAL INFORMATION

None.

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