

Probiotics and thermal processing: A review of challenges and protective strategies

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Article history:

Received: 23 July 2025

Received in revised form: 20 August 2025

Accepted: 21 August 2025

Available Online: 6 September 2025

Keywords:

Probiotics,
Thermal processing,
Microencapsulation,
Spore-formers,
Postbiotics,
Functional foods,
Food stability

Abstract

Probiotic organisms are widely acknowledged for their significant health benefits, particularly in supporting gut health, enhancing immune function, and reducing risks associated with chronic diseases. However, their broader application in thermally processed foods is restricted by their heat sensitivity, which leads to a substantial loss in viability during standard processing methods such as pasteurization, sterilization, and baking. This review presents a comprehensive overview of the mechanisms by which thermal stress impacts probiotic survival, including protein denaturation, membrane disruption, and metabolic damage. Emphasis is placed on innovative protective strategies, such as microencapsulation using advanced biopolymer systems, the use of spore-forming probiotics, and the integration of cryo- and thermo-protectants to enhance stability. Furthermore, non-thermal processing technologies—such as high-pressure processing (HPP), pulsed electric fields (PEF), ultrasound, and cold plasma—are explored as viable alternatives that preserve probiotic viability without compromising food safety. The emergence of postbiotics as heat-stable, health-promoting microbial metabolites is also discussed, offering new opportunities for functional food development. The paper concludes by highlighting future directions in formulation, regulatory guidance, and the design of next-generation probiotic and postbiotic products that align with global food system sustainability and health policy goals.

OPEN ACCESS

Citation: Awulachew, M.T. (2025). Probiotics and thermal processing: A review of challenges and protective strategies. *Letters in Food Research*, 1(4), e25057. <https://doi.org/10.26656/lifr.1.e25057>

1. Introduction

Probiotics are defined by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (Joint FAO/WHO Working Group, 2002). In recent years, the inclusion of probiotics in functional foods has grown rapidly, driven by increased consumer awareness of the role of the gut microbiota in regulating immune function, metabolic health, and the prevention of chronic diseases such as obesity, diabetes, and inflammatory bowel disease (Hill *et al.*, 2014; Markowiak and Śliżewska, 2017). Traditionally, probiotics—most commonly *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces* species—have been incorporated into dairy-based products like yogurt, kefir, and cheese, as well as in fermented vegetables and beverages.

However, there is now a growing interest in expanding probiotic applications to non-dairy and shelf-stable processed foods such as cereals, baked goods, snacks, and functional beverages. Despite this,

technological challenges remain, particularly related to the thermal sensitivity of probiotic cells. Probiotics are biologically fragile and susceptible to processing stressors, especially heat, oxygen, and low pH. Thermal processing—critical for ensuring microbial safety, shelf-life, and consumer acceptability—typically involves exposure to temperatures ranging from 60°C to over 100°C, which can result in significant loss of probiotic viability. To confer their intended health benefits, it is widely accepted that viable counts of probiotics must remain above 10^6 to 10^7 CFU/g at the time of consumption. Without adequate protective strategies, thermal treatments such as pasteurization, sterilization, baking, extrusion, and spray-drying can reduce cell viability below this threshold, rendering the product ineffective.

The increasing demand for ambient-stable probiotic-enriched functional foods—especially in regions with limited cold-chain infrastructure—highlights the need for a deeper understanding of how thermal processes impact probiotic survival and how advanced technologies can help mitigate these effects. Recent innovations such as

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microencapsulation, spray-coating, probiotic co-formulation, and the use of thermotolerant or spore-forming strains have shown promise in enhancing probiotic resistance to harsh processing conditions (Burgain *et al.*, 2011; Cook *et al.*, 2012). Furthermore, non-thermal technologies such as high-pressure processing (HPP), pulsed electric fields (PEF), ultrasound, and freeze-drying are gaining momentum as gentle alternatives that preserve both microbial viability and product quality (Patrignani and Lanciotti, 2016).

In parallel, a new frontier in probiotic science is the emergence of postbiotics—non-viable microbial cells, metabolites, or cell components that confer health benefits to the host. Postbiotics offer enhanced stability, greater safety, and easier integration into heat-processed foods, thereby overcoming many of the limitations associated with live probiotic formulations (Salminen *et al.*, 2021; Aguilar-Toalá *et al.*, 2021). Postbiotic-enriched products may prove especially valuable in clinical nutrition, pediatric care, and functional food formulations where microbial viability cannot be guaranteed during processing or storage.

This review provides a comprehensive synthesis of current knowledge on how thermal processing affects probiotic viability and functionality, the mechanisms of thermal inactivation, and the physiological responses of probiotic strains under stress. It further evaluates existing and emerging protective strategies, such as encapsulation techniques and the use of spore-forming or thermotolerant strains, and highlights non-thermal processing technologies that offer alternatives to traditional heat treatments. Finally, the review explores the functional role of postbiotics in thermally processed foods, presenting them as a promising avenue for delivering health benefits in challenging food environments.

2. Methodology

This review is based on a systematic survey of peer-reviewed articles, reviews, and book chapters published between 2010 and 2025, using databases such as PubMed, Scopus, ScienceDirect, and Google Scholar. Keywords included “Probiotics,” “Thermal processing,” “Microencapsulation,” “Spore-formers,” “Postbiotics,” “Functional foods,” and “Food stability,” with Boolean operators (*AND*, *OR*, *NOT*) to refine searches. Inclusion criteria comprised studies published in English within the specified period, reporting quantitative results, focusing on food applications of probiotics, spore-formers, or related functional components, and demonstrating practical relevance to food systems. Exclusion criteria included studies lacking quantitative data, publications prior to 2010, research focused solely on medical or clinical

applications, and non-peer-reviewed sources. Selected studies were assessed for relevance, quality, and applicability to food systems, with priority given to research addressing thermal stability, microencapsulation efficiency, postbiotic functionality, and overall food system stability. This approach ensures the review is comprehensive, current, and focused on applied food system relevance.

3. Thermal damage to probiotics

Thermal processing methods such as pasteurization (63–85°C), sterilization (>100°C), and baking (>170 °C) are detrimental to vegetative probiotic strains. Heat exposure leads to protein denaturation, lipid membrane disruption, and DNA damage, resulting in rapid loss of cell viability (Wendel, 2022; Taspinar, 2022). Specifically, *Lactobacillus acidophilus*, *L. rhamnosus*, and *Bifidobacterium* species show significant reductions in colony-forming units (CFU) at temperatures above 60°C. Protein denaturation typically begins between 65–70°C, correlating with irreversible functional loss (Kieps *et al.*, 2023).

While freeze-drying is milder than thermal drying, it still imposes severe physical stress. The formation of extracellular and intracellular ice crystals leads to mechanical damage, osmotic imbalance, and concentration of solutes, which destabilizes membranes and causes cellular leakage (Wendel, 2022). Survival rates post-freeze-drying often fall below therapeutic thresholds (<10⁶ CFU/g) when protective agents are not used. For example, *L. rhamnosus* GG survived at only ~12% when frozen in water, though this rate improved to ~15% by including trehalose and skim milk as cryoprotectants (Wang *et al.*, 2025).

Effective cryoprotectant combinations—such as trehalose, skim milk, mannitol, or sucrose—can elevate survival rates to over 80% in certain strains. However, strain responses differ, and optimizing freezing conditions, pre-freeze treatments, and drying protocols is essential. Beyond physical disruption, freeze-drying disrupts glycometabolism, reducing enzymatic activity and ATP production, which further impairs bacterial recovery post-rehydration (Noufeu, 2025).

Hence, thermal processing degrades probiotic viability through chemical and physical damage to proteins, membranes, and genetic material, while freeze-drying imposes mechanical stresses that also limit survival. Without protective strategies, viability frequently drops below the minimum effective dose by the time probiotics reach consumers.

4. Protective strategies

4.1 Microencapsulation

Microencapsulation is a widely employed and highly effective strategy for enhancing the thermal stability and overall viability of probiotic microorganisms during processing, storage, and gastrointestinal transit. This technique involves enclosing probiotic cells within a protective matrix or coating material that acts as a barrier against environmental stressors such as heat, oxygen, moisture, and gastric acids.

Various encapsulation methods are used, including spray-drying, freeze-drying, coacervation, extrusion, and emulsion-based techniques. Among them, spray-drying is the most scalable and cost-effective approach for industrial application, although it exposes the probiotics to high inlet temperatures (~150–200°C), which makes the choice of wall material crucial for cell survival. Protective carriers such as maltodextrin, gum arabic, inulin, whey protein isolate, and prebiotic fibers like fructooligosaccharides (FOS) are commonly used for their thermoprotective and synbiotic properties (Misra *et al.*, 2022; Zhou *et al.*, 2023).

For instance, *Lactobacillus reuteri* encapsulated in a rice protein–inulin gel matrix demonstrated significant thermal resistance, with a viability loss of less than 1 log CFU/g after exposure to 80°C for 10 minutes, showing the promise of plant-based encapsulants in probiotic preservation (Mudgil *et al.*, 2024). Similarly, Zhou *et al.* (2023) reported that encapsulated *Lactobacillus plantarum* in a sodium alginate–chitosan shell maintained over 80% viability after spray drying at 130°C inlet temperature.

Moreover, multi-layer encapsulation techniques, such as layer-by-layer (LbL) assembly of biopolymers, and incorporation of prebiotics or antioxidants into the microcapsules have been shown to further enhance probiotic survival. This synergistic encapsulation not only improves heat resistance but also facilitates colonization in the gastrointestinal tract and contributes to gut microbiome modulation.

Emerging technologies such as electrohydrodynamic encapsulation, 3D bioprinting, and nanocoating with bioactive compounds are under investigation to further optimize encapsulation efficiency, scalability, and targeted delivery.

Overall, microencapsulation serves as a pivotal tool for the development of functional foods containing viable probiotics, especially in products requiring thermal processing such as bakery goods, cereals, and beverages.

4.2 Spore-forming probiotics

Spore-forming probiotics, such as *Bacillus coagulans*, *B. subtilis*, and *B. clausii*, are well-suited for use in thermally processed foods due to their innate heat resistance, physical robustness, and extended shelf life. These organisms enter a dormant state by forming endospores—structures with multilayered protective coats that shield them from high temperatures (up to 100°C or more), desiccation, radiation, and extreme pH changes (Payne *et al.*, 2024).

Spore resilience is well-documented in baked foods. In a recent study, *B. subtilis* and *B. coagulans* introduced into cookies and crackers retained viability after exposure to baking temperatures ranging from 180°C to 235°C, with <2 log CFU loss for *B. subtilis* and around 3.3–4.2 log CFU reduction for *B. coagulans* across 10 months of storage (Payne *et al.*, 2025). This far outperforms non-spore-forming probiotics like *L. acidophilus*, which showed substantially higher mortality under the same conditions.

Further, *B. subtilis* strains have displayed extreme thermal tolerance, with survival after pasteurization exposure at 63–90°C in different food matrices—including milk and juice—with nearly 100% viability post-treatment (Dixit *et al.*, 2024; Payne *et al.*, 2025).

A broader evaluation of spore-forming probiotics confirmed that *B. coagulans* MTCC 5856 spores exhibit strong heat resistance, with a D-value (time to reduce one log CFU) of 35.7 min at 90°C, demonstrating exceptional thermal stability (Majeed *et al.*, 2021). Their high survivability under heat makes them prime candidates for functional ingredient applications that require pasteurization or baking.

Beyond thermal resilience, spore-formers offer significant industrial advantages: they remain stable during long-term storage under various conditions, exhibit reliable reactivation in the gut, and are generally recognized as safe (GRAS). Their widespread use in baked goods, processed dairy, sports nutrition, and synbiotic formulations reflects their versatility and industrial relevance (Payne *et al.*, 2025).

4.3 Non-thermal processing alternatives

Non-thermal technologies are gaining prominence as promising alternatives to conventional heat treatments for preserving both probiotic viability and food safety. These methods—such as high-pressure processing (HPP), pulsed electric fields (PEF), ultrasound (US), and cold plasma (CP)—inactivate spoilage and pathogenic microorganisms while minimizing structural and nutritional damage to sensitive food components, including probiotics.

4.3.1 High-pressure processing

High-pressure processing (HPP) subjects food to pressures of 400–600 MPa at ambient or mildly elevated temperatures, disrupting pathogenic cell membranes while sparing beneficial bacteria. Probiotic strains like *Lactobacillus acidophilus* and *Bifidobacterium lactis* exhibit >90% survival at 400 MPa for 5 minutes, compared to <10% survival in thermally pasteurized equivalents (Misra *et al.*, 2022; Zhou *et al.*, 2023). HPP-treated dairy and beverages retain probiotic functionality, improved sensory characteristics, and longer shelf life.

4.3.2 Pulsed electric fields

Pulsed electric field (PEF) involves applying short bursts of high-voltage electric fields (typically 20–40 kV/cm) to liquid foods. This method permeabilizes microbial membranes without causing thermal degradation. Studies have shown that *Lactobacillus plantarum* and *L. casei* maintain up to 95% viability after PEF treatment, while pathogens such as *Listeria monocytogenes* are significantly reduced (Misra *et al.*, 2022). PEF is particularly effective in fruit juices and probiotic-enriched beverages.

4.3.3 Ultrasound

Low-frequency, high-intensity ultrasound (20–100 kHz) generates cavitation bubbles that rupture microbial membranes. When optimized, ultrasound can retain 70–90% probiotic viability while improving product emulsification and bioactive compound release. Recent innovations also combine ultrasound with encapsulation to improve probiotic retention under adverse conditions (Zhou *et al.*, 2023).

This study investigated the impact of ultrasound treatment on the fermentation kinetics, viscosity, and water-holding capacity of millet-based probiotic beverages. The results indicated that ultrasound pre-treatment significantly influenced these properties, suggesting its potential in enhancing probiotic functionality in plant-based matrices (Meena *et al.*, 2024). Research published in MDPI examined how high-intensity ultrasound affects the growth and fermentation profiles of probiotics like *Lactobacillus acidophilus* and *Lactobacillus helveticus*. The study found that ultrasound amplitude notably influenced the production of organic acids, which are crucial for probiotic activity and food preservation (Bolívar-Jacobo *et al.*, 2024). A study in the Journal of Applied Microbiology explored the effects of ultrasound application on the viability of yogurt starter cultures and the sensory properties of the product. The findings suggested that ultrasound

treatment could enhance the growth of lactic acid bacteria, thereby improving the probiotic content and quality of yogurt (Tavşanlı *et al.*, 2024). These studies highlight the diverse applications of ultrasound in enhancing probiotic viability and functionality across various food matrices.

4.3.4 Cold plasma

Cold atmospheric plasma, a partially ionized gas rich in reactive species, is a cutting-edge tool for surface decontamination and microbial reduction in packaging and food surfaces. While still under exploration, cold plasma has demonstrated minimal negative effects on spore-forming and encapsulated probiotics in dry foods and coatings. It offers an eco-friendly, energy-efficient solution for extending shelf life and ensuring hygiene in functional products (Misra *et al.*, 2022).

Cold plasma treatment has demonstrated high effectiveness in inactivating spore-forming microorganisms while elucidating its underlying mechanisms. It represents a promising non-thermal technology for microbial decontamination in food processing, showing robust activity against resilient spores without compromising the quality, nutritional value, or sensory attributes of heat-sensitive food products (Ezzati *et al.*, 2025).

5. Postbiotics and emerging trends

Postbiotics refer to non-viable microbial cells, their components, or metabolic byproducts that confer health benefits to the host (Salminen *et al.*, 2021). These include short-chain fatty acids (SCFAs), bacteriocins, enzymes, peptidoglycans, teichoic acids, and other cell wall-derived compounds. Unlike live probiotics, postbiotics are stable to thermal treatments and are increasingly favored in thermally processed food products (Aguilar-Toalá *et al.*, 2018).

Studies have shown that heat-killed strains such as *Lactobacillus paracasei* and *Bifidobacterium breve* can exert immunomodulatory effects, enhance gut barrier function, and reduce inflammation (Zhou *et al.*, 2023). For example, *Lactobacillus rhamnosus* GG lysates have demonstrated protective effects against gastrointestinal pathogens and cytokine imbalance in both in vitro and animal studies (Tsilingiri *et al.*, 2012).

The use of postbiotics is growing in applications such as infant formula, functional beverages, nutraceuticals, and clinical nutrition, particularly for vulnerable populations like immunocompromised individuals (Aguilar-Toalá *et al.*, 2018; Zhou *et al.*, 2023). Their longer shelf life, consistent composition, and absence of viability requirements make them attractive for commercial food processing (Aguilar-Toalá *et al.*, 2018; Salminen *et al.*, 2021). As interest in

precision nutrition and functional foods grows, postbiotics represent a promising frontier in the development of next-generation health-promoting food ingredients (Tsilingiri *et al.*, 2012; Salminen *et al.*, 2021).

6. Challenges and protective strategies

6.1 Challenges

As the demand for probiotic-enriched and shelf-stable functional foods continues to grow, several key areas require attention to overcome the limitations imposed by thermal processing. Future research and industrial applications should focus on the following strategic directions:

6.1.1 Strain-specific thermotolerance characterization

There is a critical need to deepen the understanding of the genetic and phenotypic factors that govern heat resistance in different probiotic strains. This includes identifying naturally resilient species, engineering thermo-tolerant variants through adaptive evolution or CRISPR-based genome editing, and creating comprehensive databases of strain-specific processing thresholds.

6.1.2 Advancement in encapsulation technologies

The development of novel encapsulation systems—especially those employing intelligent, responsive biopolymers—should be prioritized. These systems should offer not only thermal protection but also targeted release mechanisms within the gastrointestinal tract. Multi-layered, synbiotic (probiotic + prebiotic) encapsulates, nanoemulsions, and hybrid protein-polysaccharide matrices represent promising avenues.

6.1.3 Integration of postbiotics into product design

As postbiotics gain traction due to their stability and safety, future formulations should emphasize their inclusion, particularly in thermally processed, long-shelf-life products. Functional evaluation of specific postbiotic compounds (e.g., SCFAs, enzymes, cell wall peptides) in human clinical trials will be essential for health claims and regulatory acceptance.

6.1.4 Adoption of scalable non-thermal technologies

High-pressure processing (HPP), pulsed electric fields (PEF), ultrasound, and cold plasma have shown potential at laboratory and pilot scale. Future directions should focus on cost reduction, energy optimization, and industrial integration of these technologies into commercial food processing lines.

6.1.5 Incorporation into climate-resilient and policy-aligned food systems

With growing interest in sustainable food systems and reducing cold-chain dependency, research should align with national and global food security policies. This includes designing heat-stable probiotic and postbiotic formulations that can be used in rural or resource-limited settings without refrigeration.

6.1.6 Consumer education and regulatory harmonization

Promoting awareness about the benefits of postbiotics and thermally protected probiotics can drive market acceptance. Parallel to this, harmonizing regulatory frameworks across regions (e.g., EU, FDA, Codex) regarding labeling, safety, and efficacy claims will support international commercialization.

6.1.7 Omics-driven insights for functionality

Integration of metagenomics, metabolomics, and transcriptomics will be critical to better understand how thermal stress alters probiotic activity and how postbiotics interact with host physiology. Such insights will facilitate the personalized design of probiotic formulations.

6.2 Perspectives

Probiotics offer significant potential for functional foods, yet their sensitivity to thermal processing remains a key challenge. Addressing strain-specific thermotolerance, developing advanced encapsulation technologies, integrating postbiotics, and adopting scalable non-thermal processing methods are critical for enhancing probiotic stability. Additionally, aligning formulations with sustainable, climate-resilient food systems, promoting consumer awareness, and harmonizing regulatory frameworks will be essential for successful market translation.

Future progress will benefit from interdisciplinary approaches combining omics-driven insights, innovative material design, and process engineering to ensure safe, stable, and effective probiotic products. By strategically bridging fundamental research with practical applications, the food industry can maximize the health benefits of probiotics and postbiotics while supporting global food security and resilient functional food systems.

Conclusion

Thermal processing remains one of the most significant hurdles in the formulation of probiotic-enriched functional foods due to the high susceptibility of probiotic strains to heat-induced inactivation. Despite these limitations, advances in microencapsulation,

particularly with synbiotic and multi-layer protective matrices, have demonstrated promising results in preserving cell viability under severe conditions. The deployment of spore-forming strains like *Bacillus coagulans* further enhances resilience in baked and sterilized products. In parallel, non-thermal preservation methods such as HPP and PEF are gaining commercial relevance for their dual ability to ensure safety and retain probiotic functionality.

Importantly, the field is witnessing a paradigm shift toward postbiotics, which offer robust thermal stability, defined chemical profiles, and established health benefits—making them ideal for integration into shelf-stable, industrial food products. Moving forward, synergizing these strategies with real-time food industry needs and aligning with international food and nutrition policy frameworks will be crucial. Continued research should focus on strain-specific thermotolerance, functional characterization of postbiotic compounds, and the development of cost-effective delivery systems. Such innovation will enable the production of next-generation probiotic and postbiotic products that are safe, effective, and accessible globally, especially in settings lacking cold-chain infrastructure.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The author sincerely acknowledges the collective efforts of the scientific community whose valuable contributions have provided the foundation for this review. I am also grateful for the institutional and academic environment that supported the development of this work. This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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