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Postbiotics as Biochemical Agents in Functional Foods: Mechanisms, Stability, and Health Implications

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ABSTRACT

Postbiotics, defined as preparations of inanimate microorganisms and their metabolites that confer health benefits to the host, have emerged as promising agents in functional food science and biomedicine. Unlike probiotics, postbiotics offer enhanced safety, improved stability, and compatibility with various food processing technologies. This review explores the biochemical mechanisms underlying postbiotic activity, focusing on key metabolites such as short-chain fatty acids, bioactive peptides, and microbial cell wall components. These compounds interact with host receptors to modulate immune responses, reinforce intestinal barrier integrity, and influence systemic metabolic regulation. The stability of postbiotics under thermal and nonthermal processing conditions is critically examined, highlighting factors such as temperature, moisture, oxygen exposure, and food matrix composition that influence bioactivity retention. Analytical techniques, including chromatography, spectroscopy, immunoassays, and *in vitro* gastrointestinal models, are discussed as tools for evaluating composition, functionality, and bioavailability. The manuscript also reviews the application of postbiotics across dairy, beverages, bakery products, snacks, and processed meats, demonstrating their potential in promoting gut health, metabolic balance, and immune modulation. Strategies such as microencapsulation, advanced packaging, and optimized formulation are emphasized for enhancing stability and delivery. Finally, the paper outlines future research directions involving clinical validation, precision nutrition, and the integration of omics technologies to support personalized dietary interventions. By bridging biochemistry, food technology, and health science, this work provides a comprehensive framework for advancing the development and application of postbiotics in functional food systems.

KEYWORDS

Postbiotics, functional foods, short chain fatty acids, gut microbiota, intestinal barrier function, immune modulation, food processing stability, bioactive compounds, nutritional biochemistry

INTRODUCTION

The growing interest in gut health and its relationship to systemic well-being has intensified the exploration of microbiota-derived compounds, particularly postbiotics. Defined by Salminen *et al.*[1] as "a preparation of inanimate microorganisms and/or their components that confers a health benefit on the

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host", postbiotics differ fundamentally from probiotics by offering advantages in terms of safety, shelf-stability, and ease of incorporation into processed foods[1]. Their relevance is especially significant in vulnerable populations where the use of live bacteria may pose risks, and in industrial applications where microbial viability is often compromised during processing[1]. These properties make them ideal for use in functional food and public health applications[1].

Numerous studies have elucidated the composition and biological functions of postbiotic compounds. For instance, Short-Chain Fatty Acids (SCFAs) such as butyrate, acetate, and propionate produced during microbial fermentation have demonstrated roles in maintaining epithelial integrity, modulating immune responses, and supporting metabolic health[1,2]. Bioactive peptides and microbial cell wall fragments, including lipoteichoic acid and peptidoglycans, also contribute significantly to immunomodulatory and anti-inflammatory effects by interacting with host pattern recognition receptors such as Toll-Like Receptors (TLRs) and NOD-like receptors (NLRs)[1,2]. Moreover, postbiotic preparations have been shown to influence gut microbiota composition indirectly and support intestinal barrier function, contributing to gastrointestinal homeostasis[1,2].

Despite these promising findings, literature addressing the stability of postbiotic compounds during thermal and non-thermal food processing, as well as their functional retention during storage, remains fragmented and underdeveloped[3-5]. Recent reviews have called for a more integrative understanding that bridges the biochemical functionality of postbiotics with food technology and health application domains[2]. This review aims to fill that gap by synthesizing existing knowledge on the biochemical mechanisms underlying postbiotic activity, assessing how food processing and storage conditions affect their stability, and evaluating their health implications based on current evidence.

Furthermore, it discusses emerging analytical tools for postbiotic characterization, including chromatographic, spectroscopic, and immunological methods[2], and explores the role of postbiotics in various processed food categories such as dairy, beverages, and bakery products[2]. Innovations in encapsulation and delivery systems also offer new directions for improving postbiotic bioavailability[2], while advances in microbiome research are paving the way for precision nutrition strategies using postbiotic-based interventions[2].

RESEARCH METHODLOGY AND LITERATURE SYNTHESIS

Overview: This section outlines the methodology adopted in developing a comprehensive narrative review of postbiotic compounds, emphasizing their biochemical mechanisms, functional stability in processed foods, and associated health outcomes. A structured search strategy was utilized across multiple academic databases to identify peer-reviewed studies published between 2014 and 2025[3]. Analytical emphasis was placed on SCFAs, peptides, and microbial fragments, with data extraction focused on their stability during food processing and bioactivity *in vitro* and *vivo*[3]. Specific analytical techniques such as HPLC, ELISA, and omics-based approaches were reviewed[3]. The methodology provided a robust foundation for integrating multidisciplinary findings[3].

Literature search and selection strategy: A narrative literature review was employed to explore the biochemical, technological, and clinical dimensions of postbiotics. Searches were conducted in PubMed, Web of Science, Scopus, and Google Scholar using terms like "Postbiotics", "Short-chain fatty acids", "Microbial metabolites", "Functional food stability", and "Gut barrier integrity". Included studies were peer-reviewed, full-text English articles from 2014 to 2025 focusing on postbiotic characterization, health effects, or stability. Abstracts and references were screened, resulting in 87 eligible studies for full review[3,4].

Table 1: Summary of analytical techniques used to evaluate postbiotics

Analytical method	Target compound(s)	Application area	Relevant citations
HPLC/GC-MS	SCFAs, peptides	Quantification and profiling	Thorakkattu *et al.*[3] and
ELISA	Cytokines, immune markers	Immunomodulatory assessment	Homayouni-Rad *et al.*[4]
NMR/LC-MS/MS	Organic acids, peptides	Structural characterization	
SHIME/TIM-1	Postbiotic fractions	Simulated gut bioavailability	
Metagenomics/qPCR	Microbial community shifts	Gut microbiota response analysis	
Omics (Metabolomics)	Functional metabolites	Mechanism elucidation	

The table summarizes the key analytical techniques used to evaluate postbiotics in food and biological systems. It highlights the target compounds, areas of application, and corresponding sources for each method. This categorization provides a comparative framework for selecting appropriate tools in postbiotic research and analysis

Data extraction and analytical criteria: From the selected literature, data were categorized based on compound type, food matrix, processing method, analytical technique, stability, and observed bioactivity. Studies using GC-MS, HPLC, ELISA, and *in vitro* digestion simulations such as SHIME® were prioritized due to their quantitative reliability and biological relevance[3,4]. The review particularly emphasized food systems, including dairy, cereal-based products, and beverages. Studies showing immune, metabolic, or gut-barrier modulation through defined biochemical mechanisms were also prioritized[3,4]. Analytical rigor and reproducibility were applied as secondary filters[4] (Table 1).

Organization and synthesis of evidence: Extracted data were synthesized thematically into domains: biochemical composition and function, stability during processing, analytical tools, and health-related bioactivity. These domains integrated findings from nutritional biochemistry, clinical nutrition, and food technology. Data were structured into summary tables and figures to facilitate comparison across compound types and processing conditions. This approach ensured a multifactorial understanding of postbiotic applications and challenges.

BIOCHEMICAL INSIGHTS, FUNCTIONAL STABILITY, AND HEALTH APPLICATIONS OF POSTBIOTICS

Overview: This section presents a comprehensive analysis of the biochemical mechanisms and functional relevance of postbiotics. It explores the production of key compounds such as SCFAs, bioactive peptides, and microbial cell wall components, and their interactions with host systems. The discussion highlights their roles in reinforcing gut barrier function, modulating immune responses, and regulating metabolic pathways. Stability under various food processing and storage conditions is critically examined. Analytical methods, including chromatographic and cell-based assays, support the functional claims. Overall, the findings emphasize the potential of postbiotics in advancing functional food science and health applications.

Biochemical mechanisms of postbiotic action: As defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP), postbiotics are "preparations of inanimate microorganisms and/or their components that confer a health benefit on the host"[5]. Unlike live probiotics, postbiotics are more stable during processing and storage[5,6]. Their beneficial effects are mediated through bioactive molecules produced during microbial fermentation or released following cell inactivation[6].

Key postbiotic compounds and their production: Short-chain fatty acids (SCFAs), particularly acetate, propionate, and butyrate, are produced by microbial fermentation of dietary fibers and have been widely studied for their health benefits[7]. In the gut, microbial genera such as Lactobacillus and Clostridium convert pyruvate into acetyl-CoA via pathways like Wood–Ljungdahl and acrylate; acetyl-CoA is then converted to butyrate-by-butyrate kinase, while propionate follows alternate biosynthetic routes[7]. Beyond SCFAs, fermentation also generates bioactive peptides, bacteriocins, vitamins, and microbial cell wall components like peptidoglycans and lipoteichoic acids, all contributing to postbiotic activity. Such molecules interact with host immune receptors (e.g., TLRs, NODs), modulating inflammatory and barrier responses[7,8].

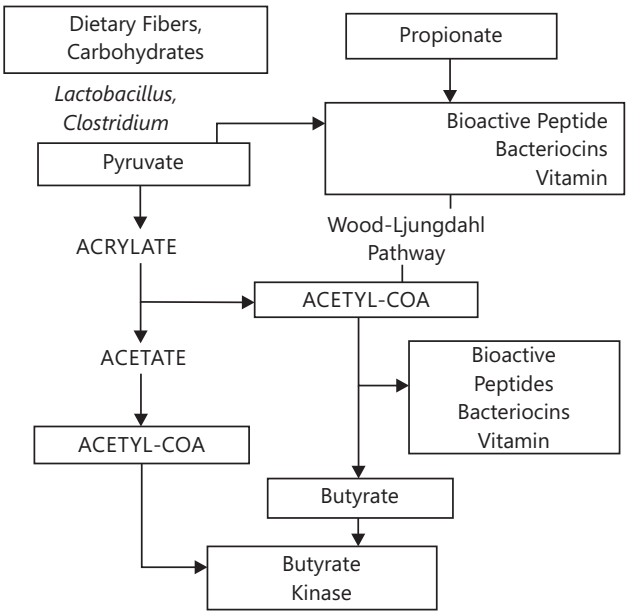

Fig. 1: Microbial metabolic pathways involved in the production of short chain fatty acids and postbiotic compounds from dietary fibers and carbohydrates (self-generated)

This diagram describes microbial fermentation pathways that convert dietary fibers into postbiotic compounds. Pyruvate and acetyl-CoA are central intermediates in producing SCFAs via the acrylate and Wood Ljungdahl pathways. These pathways also contribute to the formation of bioactive peptides, bacteriocins, and vitamins

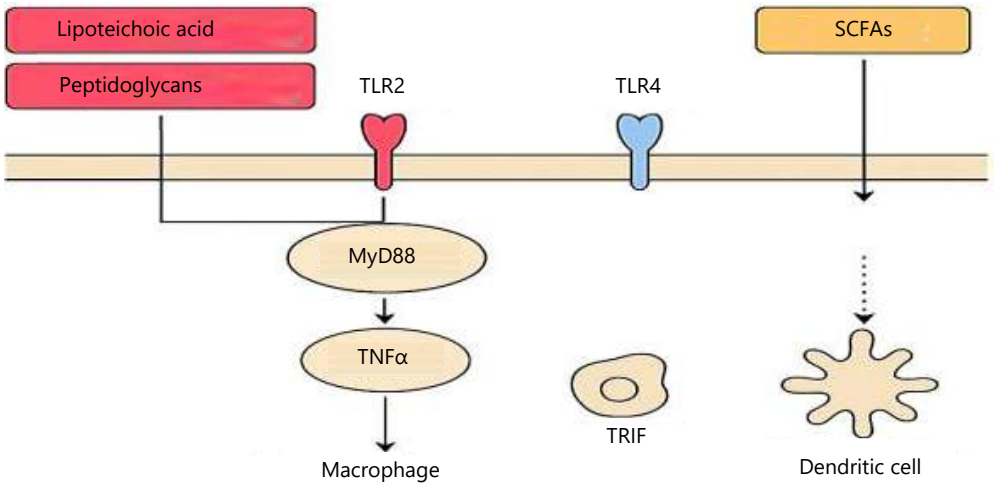

Fig. 2: Immunomodulatory mechanisms of postbiotics via pattern recognition receptors (self-generated)

Schematic representation of postbiotic signaling through Toll-like receptors (TLRs). Peptidoglycans and lipoteichoic acid activate TLR2, inducing MyD88-dependent TNF-α production and macrophage activation. SCFAs modulate immune responses by influencing dendritic cell function and potentially altering TLR4 signaling pathways

As illustrated in Fig. 1, fermentation begins with the microbial breakdown of complex carbohydrates by genera such as Lactobacillus and Clostridium, yielding pyruvate as a central intermediate. From pyruvate, different metabolic routes are engaged: Acetate and acetyl-CoA are generated via the acrylate and Wood-Ljungdahl pathways, while acetyl-CoA is further converted to butyrate through the action of butyrate kinase. Propionate, meanwhile, feeds into the Wood Ljungdahl pathway, supporting SCFA synthesis and contributing to the generation of additional bioactive compounds[7,8].

Beyond SCFAs, these fermentation pathways also result in the biosynthesis of other health-promoting metabolites, including bioactive peptides, bacteriocins, and vitamins. These compounds may arise from microbial metabolism directly or through secondary pathways such as the proteolytic degradation of dietary and host proteins[8]. Together, these molecular products form the functional basis of postbiotics, integrating microbial activity with host physiology[8].

Mechanisms of interaction with the host: A crucial mechanism of postbiotic action involves enhancement of the intestinal barrier. Short-chain fatty acids, particularly butyrate, strengthen epithelial tight junctions, reducing permeability and preventing microbial translocation that can lead to inflammation[9].

As depicted in Fig. 2, Postbiotics also exert immunomodulatory effects through microbial components such as lipoteichoic acid and peptidoglycans, which activate pattern recognition receptors like TLR2 via the MyD88/NFκB pathway, leading to controlled macrophage activation and TNF-α production . Additionally, SCFAs bind to receptors GPR43 and GPR109A on dendritic cells, influencing immune tolerance and resolving inflammation[9,10].

The anti-inflammatory role of postbiotics extends to modulating cytokine profiles, reducing pro-inflammatory mediators, and increasing IL-10, and providing antioxidant protection via certain peptides and organic acids[10].

Moreover, SCFAs like butyrate serve as key metabolic substrates for colonocytes and influence systemic glucose and lipid metabolism through endocrine and epigenetic pathways[10].

Emerging research supports the role of microbial cell wall–derived postbiotics in gut barrier repair. Peptidoglycan and lipoteichoic acid from Lactobacillus species have been shown to upregulate tight junction proteins (e.g., ZO-1, occludin) *in vitro* and improve barrier integrity *in vivo*[10].

Postbiotics impact host physiology through barrier enhancement, immune modulation, anti-inflammatory and antioxidant activity, and metabolic regulation, mediated by SCFAs and structural microbial components interacting via key signaling receptors[10].

Modulation of gut microbiota and barrier function: Though postbiotics are non-viable, they can indirectly shape the composition and activity of the resident gut microbiota[11]. The SCFAs like butyrate serve as energy sources for butyrate-producing bacteria, fostering their growth through cross-feeding mechanisms[11]. Postbiotic components also modulate microbial metabolic output, enhancing beneficial metabolite production and suppressing harmful compounds[11]. Some exert antimicrobial effects, suppressing pathogens and promoting microbiota balance[11,12].

As described in Fig. 3, Postbiotics play a key role in intestinal barrier integrity. Butyrate energizes colonocytes and upregulates tight junction proteins such as occludin and zonula occludens 1 (ZO-1), reinforcing barrier function[11,12]. The SCFAs additionally stimulate mucin secretion from goblet cells and inhibit epithelial apoptosis[12]. Other metabolites including bioactive peptides and organic acids enhance barrier integrity via signals that support epithelial survival, differentiation, and mucus production[12]. For instance, Lactobacillus-derived peptides have been shown to upregulate ZO-1 and occludin *in vitro*[12].

Immunomodulatory and metabolic effects: Postbiotics exert multifaceted effects on immunity and metabolism. Microbial structural components (lipoteichoic acid, peptidoglycan) engage innate immune receptors such as TLR2, TLR4, and NOD2 on dendritic cells, triggering cascades that balance pro-inflammatory (IL-6) and anti-inflammatory (IL-10) cytokine responses[13].

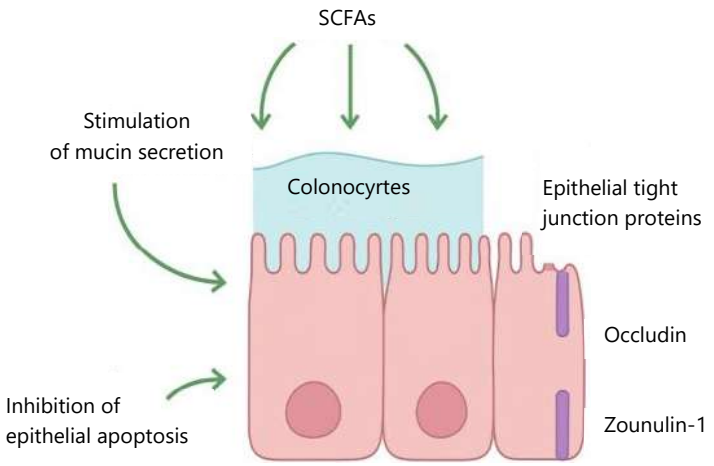

Fig. 3: Mechanisms of SCFA mediated intestinal barrier protection (self-generated)
This diagram illustrates how SCFAs act on colonocytes to stimulate mucin secretion, enhance the expression of tight junction proteins such as occludin and zonulin 1, and inhibit epithelial apoptosis. These actions contribute to maintaining gut barrier integrity and promoting intestinal health

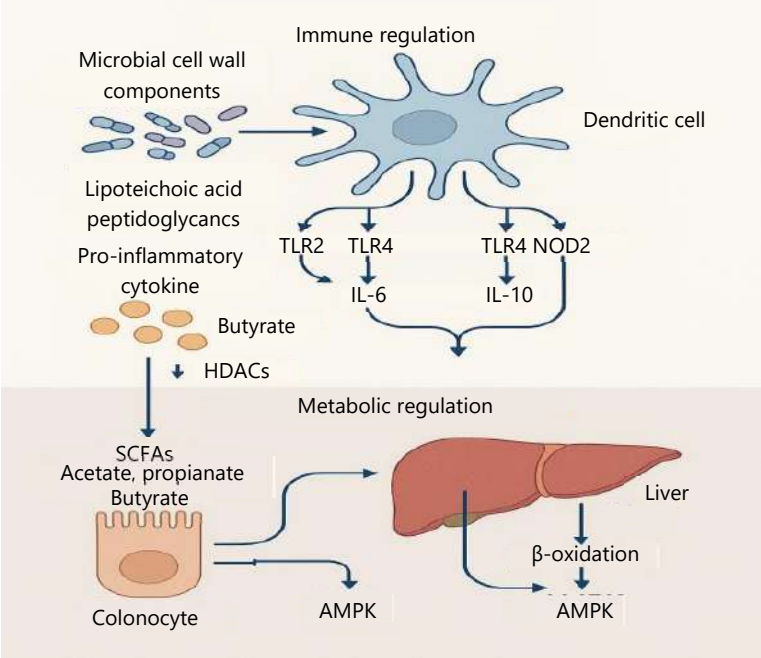

Fig. 4: Systemic effects of postbiotics on immune and metabolic regulation (self-generated)
The upper half illustrates how microbial wall components engage TLR2, TLR4, and NOD2 on dendritic cells to induce IL 6 and IL 10. The lower half shows how SCFAs act on colonocytes and hepatocytes, modulating AMPK signaling and beta oxidation, while butyrate inhibits HDACs to support immune metabolic crosstalk

The SCFAs further modulate immunity by inhibiting histone deacetylases, altering gene expression to support anti-inflammatory phenotypes and regulatory T cell development[13]. They influence immune cell behavior via receptors such as GPR41, GPR43, and GPR109A[13,14], while bolstering antioxidant defenses[13,14].

Metabolically, SCFAs contribute significantly: butyrate fuels colonocytes and maintains barrier health; acetate and propionate regulate hepatic β-oxidation and activate AMPK, improving lipid metabolism and glucose homeostasis[14]. Activation of AMPK reduces lipogenesis and enhances fat catabolism[14].

The dual effects depicted in Fig. 4, immune modulation via cytokine signaling and metabolic enhancement through SCFA-mediated energy regulation, highlight the therapeutic relevance of postbiotics. Their ability to fine-tune immune and metabolic pathways positions them as attractive candidates for interventions in chronic inflammatory diseases, metabolic syndrome, and functional food applications[14].

Table 2: Effects of Thermal and non-thermal food processing techniques on the stability of key postbiotic compounds

Processing method	Conditions	Postbiotic component	Observed effect	Citations
Pasteurization	60–85°C for 15-30 min	SCFAs, peptides	Partial degradation of heat-sensitive peptides	Tlais *et al.*[15] and Palomino *et al.*[16]
Sterilization	≥121°C, 15-20 min	Peptidoglycans, SCFAs	Structural alteration of cell wall fragments	
High-pressure processing	400–600 MPa, room temperature, 1-10 min	SCFAs, peptides	Good retention of bioactivity	
Pulsed electric fields	20–50 kV/cm pulses	SCFAs	Minimal impact on SCFA profile	
Irradiation	1-10 kGy	Peptides, organic acids	Dose-dependent degradation	
Freeze-drying	-50°C to -80°C under vacuum	Peptides, SCFAs	High stability, minimal degradation	
Spray-drying	140-200°C inlet, short time	Peptides, SCFAs	Moderate loss, especially heat-sensitive compounds	
In situ fermentation	25-42°C, variable pH and time	All classes	Active production of stable postbiotic compounds	

The table summarizes the effects of thermal and non-thermal processing techniques on the stability of key postbiotic compounds. It directly complements section 3.2.1 by linking specific processing methods to their impacts on compound integrity, bridging theory and application

Together, these immune and metabolic effects highlight postbiotics therapeutic potential in managing inflammation, metabolic syndrome, and their incorporation into functional foods.

Impact of food processing technologies: Food processing techniques are essential for ensuring safety, extending shelf life, and enhancing the palatability of food products. However, many of these processes involve conditions that could potentially compromise the integrity and bioactivity of postbiotic compounds.

Thermal processing, such as pasteurization, sterilization, and cooking, is widely used to eliminate pathogenic microorganisms and inactivate enzymes. While postbiotics, by definition, are non-viable, elevated temperatures can still affect the stability of their bioactive molecules. For instance, prolonged or high-temperature heating can lead to the degradation of certain short-chain fatty acids (SCFAs), the breakdown of bioactive peptides, and structural alterations in cell wall components like peptidoglycans[15]. Studies have shown varying degrees of stability for different postbiotic compounds under thermal stress[15]. The impact depends on the composition of the postbiotic preparation, the temperature and duration of heat treatment, and the surrounding food matrix[15].

Non-thermal processing technologies have gained increasing attention as an alternative to thermal processing to preserve the bioactivity of postbiotic compounds. High-Pressure Processing (HPP), which utilizes high hydrostatic pressure to inactivate microorganisms, generally has a less severe impact on covalent bonds compared to thermal treatments[15,16]. Studies suggest that HPP may better preserve the bioactivity of certain postbiotic compounds[15,16]. Pulsed Electric Fields (PEF) and irradiation are other non-thermal techniques that primarily target microbial viability but may have varying effects on the stability of specific postbiotic compounds[15,16]. Some research suggests that PEF has minimal impact on SCFA profiles, while irradiation effects are dose-dependent[15,16].

Drying and dehydration processes, such as freeze-drying and spray-drying, reduce water activity and preserve foods but can also influence postbiotic stability[15,16]. The removal of water may concentrate solutes and promote oxidative reactions or physical changes in postbiotic compounds. The drying method and final water activity level significantly affect bioactivity retention[15,16]. Freeze-drying is generally superior for preserving the structure and function of biological materials[16]. Encapsulation techniques are often used during drying to protect sensitive postbiotic compounds[16].

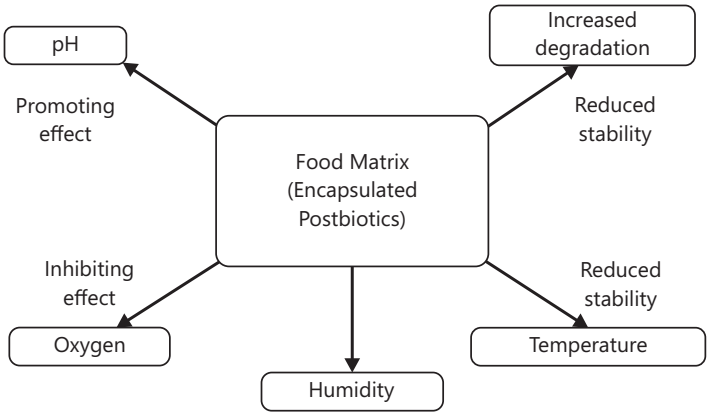

Fig. 5: Influence of food matrix and storage conditions on postbiotic stability (self-generated)
The diagram illustrates how intrinsic factors like pH and oxygen, and extrinsic conditions such as temperature and humidity, affect the stability of encapsulated postbiotics. pH may have a promoting effect, while oxygen inhibits stability. Temperature and humidity contribute to increased degradation and reduced stability. Effective encapsulation and storage are essential to preserve postbiotic functionality

Other processing techniques, such as homogenization, fermentation (*in situ* production within food matrices), and preservative addition, also influence postbiotic stability. Shear forces during homogenization may compromise cellular fragment integrity, while preservatives might interact with postbiotic molecules. Controlling fermentation parameters; temperature, pH, time is essential when postbiotics are generated *in situ* to maximize production and ensure downstream stability[16]. The timing of postbiotic formation relative to other processing steps is also critical[16].

Table 2 presents a comparative analysis of thermal and non-thermal food processing techniques and their effects on the structural integrity and bioactivity of key postbiotic compounds, including Short-Chain Fatty Acids (SCFAs), peptides, and microbial cell wall fragments. It delineates specific processing parameters and outlines the extent of compound degradation or retention under each condition. The table serves to substantiate Section 3.2.1 by providing empirical insights into how different processing methods influence postbiotic stability in functional food systems[16].

Influence of food matrix and storage conditions: The stability of encapsulated postbiotics within food products is significantly influenced by the food matrix composition and environmental storage conditions. As illustrated in Fig. 5, factors such as pH, oxygen exposure, humidity, and temperature interact to determine the degradation rate or preservation of functional bioactive compounds[17].

Food matrix composition: The physicochemical environment provided by the food matrix plays a dual role. For instance, pH can have a promoting effect, enhancing postbiotic activity or stability under optimal conditions. Conversely, exposure to oxygen typically exerts an inhibiting effect, fostering oxidative degradation, especially in sensitive molecules such as short-chain fatty acids or peptides[17].

Environmental parameters: Among the key external conditions, elevated temperature and high humidity are critical destabilizers. Temperature accelerates chemical degradation processes, while humidity increases water activity, which can drive hydrolysis and other destabilizing reactions[17,18].

Oxygen and packaging: Oxidative stress due to oxygen exposure can compromise the chemical integrity of postbiotic components. Oxygen not only inhibits functional stability but may also catalyze unwanted interactions with matrix components[17,18]. To mitigate this, advanced packaging strategies such as oxygen scavengers and modified atmosphere packaging (MAP) are essential for extending shelf life and protecting bioactivity[18].

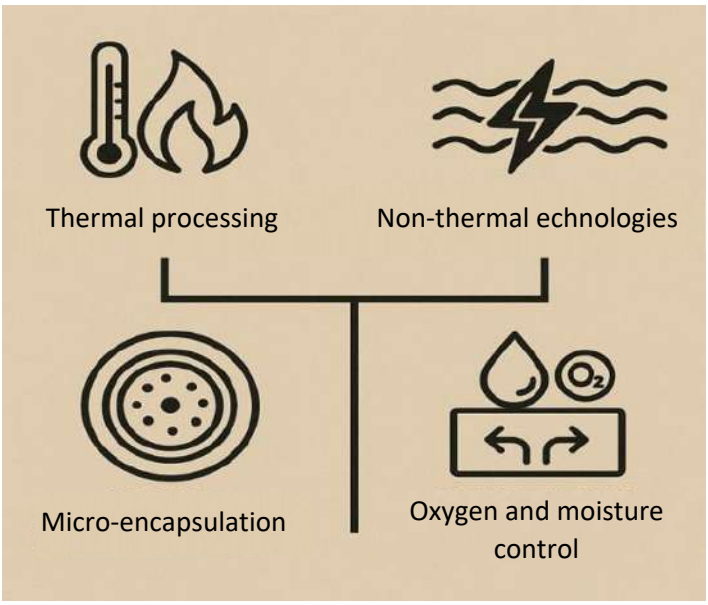

Fig. 6: Technological strategies to enhance postbiotic stability (self-generated)
This diagram shows four key methods: Termal processing, nonthermal technologies, microencapsulation, and oxygen/moisture control used to preserve postbiotic stability. These approaches help maintain bioactivity during processing, storage, and delivery

Storage parameters such as temperature, humidity, and light exposure play critical roles in the shelf life and functional retention of postbiotics in processed foods. Elevated temperatures accelerate degradation reactions, high humidity increases water activity, and UV light promotes oxidation in sensitive compounds. Therefore, storage recommendations like refrigeration, moisture control, and light protection should be clearly stated on product packaging to maintain postbiotic functionality[18].

Strategies for enhancing postbiotic stability: The stability of postbiotics is pivotal for ensuring efficacy, especially when incorporated into functional foods or pharmaceuticals. Several strategies have been optimized to protect them during processing, storage, and gastrointestinal transit.

Thermal processing and tyndallization: Mild heat treatments like pasteurization and tyndallization inactivate microbial cells while preserving bioactives. Heat killed preparations have shown comparable immunomodulatory effects to live probiotics[19].

Non thermal technologies: Approaches such as high pressure processing (HPP), pulsed electric fields, and cold plasma maintain structural integrity of postbiotic compounds without significant degradation[19].

Water activity and moisture control: Reducing water activity via dehydration or encapsulation enhances shelf life by limiting enzymatic and oxidative reactions[19,20]. Encapsulation with biopolymers like alginate or chitosan improves resilience to environmental stress[19,20].

As shown in Fig. 6, key strategies include: Thermal methods (pasteurization/tyndallization), non thermal technologies (HPP, PEF), microencapsulation with protective biopolymers, and moisture/oxygen control (e.g., MAP). These approaches help preserve the structural integrity and functional bioactivity of postbiotic compounds[19,20].

Formulation and carrier matrices: Integrating postbiotics into matrices such as dairy products, cereals, and functional beverages enhances their stability and bioavailability[20]. Matrix composition is critical for protecting postbiotics during digestion and targeting release[20].

Freeze drying and spray drying: These drying methods effectively stabilize postbiotics by controlled moisture removal, preserving bioactivity. Freeze dried formulations especially retain immunomodulatory capacity better than other drying methods[20].

Overall, optimizing stability strategies ensures postbiotics retain bioactivity through processing and storage, enhancing their health benefits and commercial viability.

Analytical methods for postbiotic assessment: The accurate assessment of postbiotic content, composition, and functionality is essential for quality control and the validation of health claims. Analytical methodologies must be robust, reproducible, and suitable for complex matrices.

Chromatographic techniques: Advanced chromatographic methods such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) are routinely employed to quantify postbiotic compounds, including short-chain fatty acids (SCFAs), peptides, and organic acids. These techniques offer high resolution and sensitivity, allowing the separation and identification of low-abundance metabolites in complex food matrices[21].

Spectroscopic and mass spectrometry-based methods: Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS), particularly when coupled with chromatographic separation (e.g., LC-MS/MS), provide structural elucidation of postbiotic compounds. These tools are especially useful for detecting and characterizing novel bioactive molecules and fermentation byproducts[21].

Immunoassays and cell-based bioassays: Enzyme-linked immunosorbent assays (ELISA) and reporter gene assays are used to evaluate the immunomodulatory properties of postbiotics. These methods help quantify cytokine production or Toll-like receptor (TLR) activity, providing functional insights into postbiotic-host interactions[21].

***In vitro* gastrointestinal models:** Simulated digestion systems like SHIME (Simulator of the Human Intestinal Microbial Ecosystem) or TIM-1 (TNO Intestinal Model) help assess the bioavailability and stability of postbiotics under physiological conditions[21,22].

Microbial and molecular techniques: Quantitative PCR and metagenomics are used to monitor microbial shifts in response to postbiotic treatment. Although postbiotics do not contain viable cells, their administration can modulate gut microbiota indirectly[21,22].

Omics approaches: Metabolomics, proteomics, and transcriptomics enable comprehensive profiling of postbiotic products and their effects on host systems. These holistic tools are instrumental in uncovering novel mechanisms of action and identifying potential biomarkers of efficacy[22].

As illustrated in Fig. 7, the analytical pipeline outlined in Section 3.2.4 is visually presented, highlighting the multi-step process used to assess the composition, stability, and bioactivity of postbiotics in food systems. The process begins with sample preparation, including key steps like extraction and filtration to isolate postbiotic compounds from complex matrices. Next, chromatographic techniques such as HPLC and GC-MS enable quantification of key compounds like SCFAs and peptides with high sensitivity. This is followed by spectroscopic and molecular assays (e.g., NMR, ELISA, qPCR) to characterize structure and detect immunological activity. The final stage includes functional assays, such as TLR activity and cytokine production tests, to assess the bioactivity and health relevance of postbiotics at the cellular level[22].

Collectively, these analytical methods ensure rigorous evaluation of postbiotic products, facilitating their standardization, regulatory approval, and integration into health-promoting interventions.

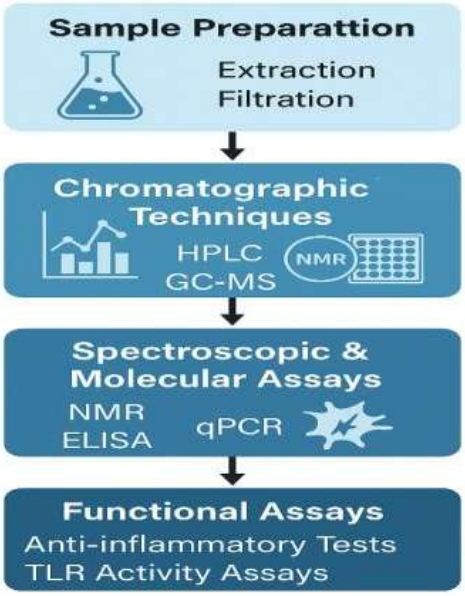

Fig. 7: Analytical pipeline for postbiotic assessment (self-generated)

This diagram illustrates the stepwise analytical process for assessing postbiotics, beginning with sample preparation and chromatographic techniques. It progresses through spectroscopic and molecular assays and concludes with functional bioactivity testing. Each step ensures precise evaluation of composition, stability, and health relevance

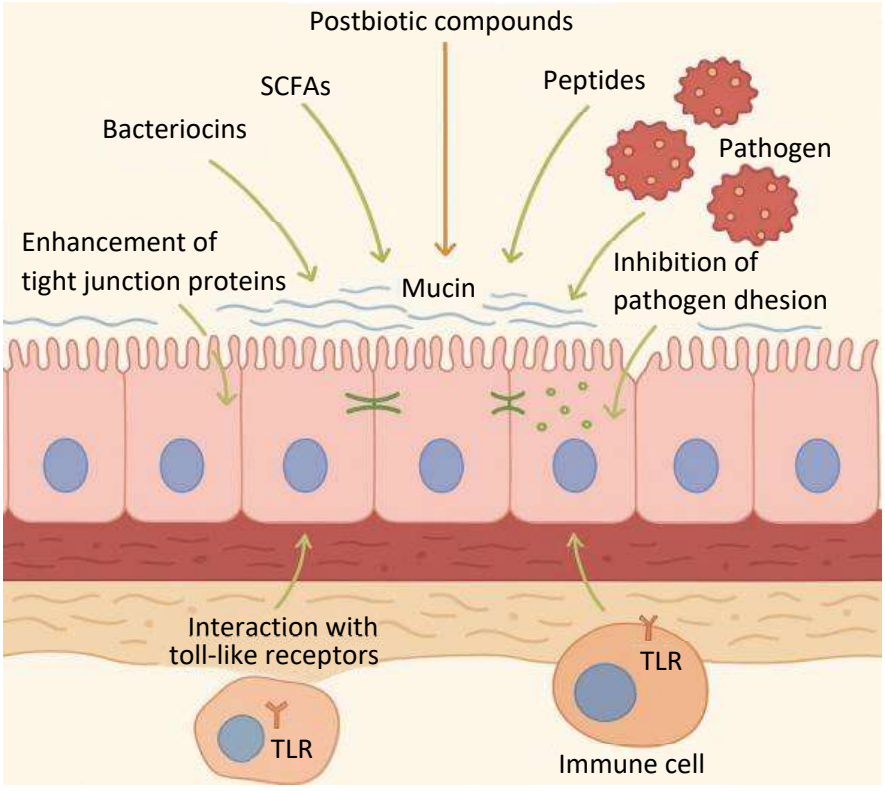

Fig. 8: Postbiotic support of gastrointestinal barrier function (self-generated)

This diagram illustrates how postbiotics like SCFAs, peptides, and bacteriocins enhance mucin production, reinforce tight junctions, and block pathogen adhesion. They also engage Toll-like receptors to support immune signaling and maintain gut barrier integrity

Impact on gastrointestinal health: Postbiotics exert a significant influence on gastrointestinal (GI) health through multiple mechanisms, including modulation of the gut microbiota, enhancement of intestinal barrier integrity, and anti-inflammatory actions. As non-viable microbial products or metabolic byproducts,

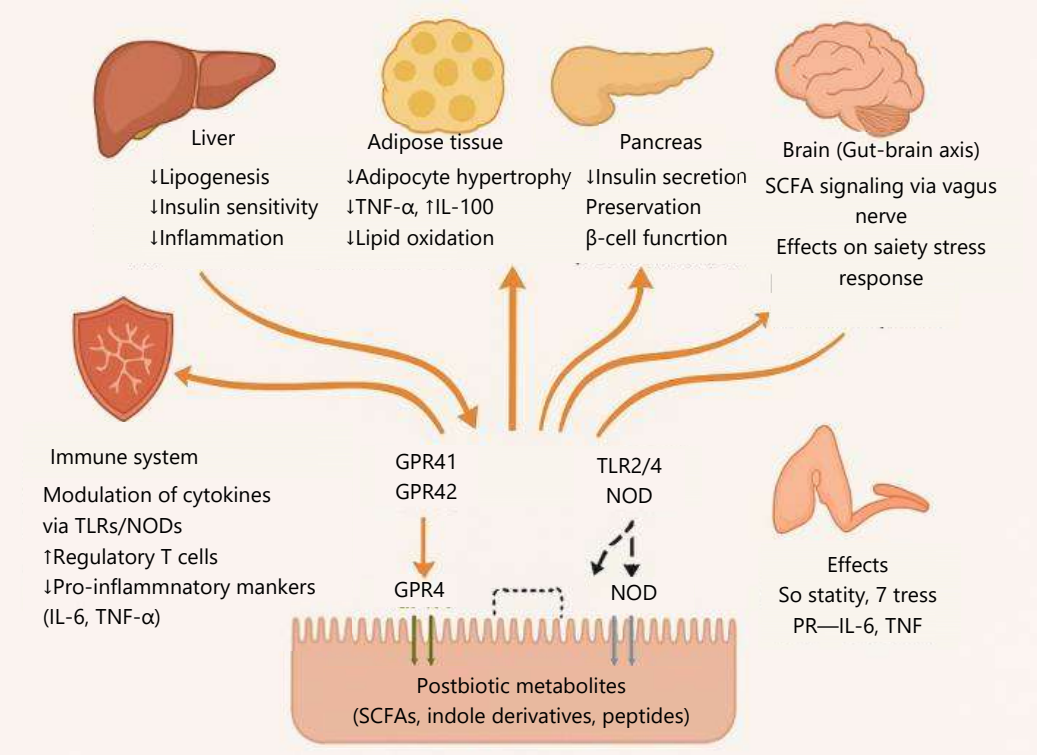

Fig. 9: Systemic health effects of postbiotics via gut–liver–immune–brain axes (self-generated)
This diagram illustrates how postbiotic metabolites such as SCFAs, indole derivatives, and peptides act through gut receptors (e.g., GPR41, TLRs) to influence key metabolic and immune organs. They improve insulin sensitivity, reduce inflammation, modulate cytokines, and affect brain signaling related to satiety and stress

postbiotics provide benefits while mitigating certain risks associated with administering live microorganisms, particularly in vulnerable populations such as infants, the elderly, or immunocompromised individuals[23].

One of the key functions of postbiotics in GI health is the reinforcement of the intestinal barrier. Postbiotic compounds such as short-chain fatty acids (SCFAs), especially butyrate, play a vital role in maintaining epithelial integrity, stimulating mucin production, and enhancing tight junction protein expression[23].

As illustrated in Fig. 8, a cross-section of the intestinal epithelium shows how SCFAs, bacteriocins, and bioactive peptides contribute to tight junction reinforcement, mucin secretion, and inhibition of pathogen adhesion, respectively. Postbiotics also interact with Toll-like receptors (TLRs) located on epithelial and immune cells, triggering beneficial immune signaling cascades. These actions preserve intestinal barrier integrity, promote mucosal immunity, and reduce inflammation and pathogen colonization[23,24]

Additionally, heat-killed probiotic strains often classified as postbiotics have demonstrated gastrointestinal benefits comparable to or exceeding those of live probiotics, particularly in reducing intestinal permeability and inflammation. Their inclusion in fermented foods further supports GI health through the combined effects of bioactive metabolites and functional nutrients[24].

Role in metabolic and immune health: Beyond gastrointestinal benefits, postbiotics exert promising effects on metabolic regulation and immune modulation. Several fermentation-derived metabolites including SCFAs, indole derivatives, and exopolysaccharides, exert systemic effects on glucose metabolism, lipid regulation, and immune homeostasis[25]. In metabolic health, postbiotics have demonstrated anti-obesity and antidiabetic effects through modulation of adipogenesis, insulin sensitivity, and lipid metabolism. For instance, specific postbiotics from Lactobacillus strains reduce body weight, hepatic fat, and insulin resistance markers in animal models[25]. These effects are partially attributed to SCFA-mediated activation of G-protein-coupled receptors and inhibition of histone deacetylases, which regulate energy metabolism and inflammation[25].

Table 3: Postbiotic applications across processed food categories

Food category	Example products	Common postbiotic components	Functional benefits	Citations
Dairy	Yogurt, kefir, fermented milk	SCFAs, peptides, heat-killed LAB	Gut microbiota modulation, barrier support, anti-inflammation	Aguilar-Toalá *et al.*[27] and Russo *et al.*[28]
Functional beverages	Kombucha, postbiotic-infused drinks	SCFAs, organic acids, antioxidants	Immune support, metabolic regulation, digestive health	
Cereal/Bakery	Postbiotic-enriched bread, snacks	Peptides, SCFAs, exopolysaccharides	Prebiotic-like effects, gut barrier reinforcement, and anti-obesity	
Snacks	Functional bars, chips, granola	Heat-stable peptides, bacteriocins	Immune function, glycemic control, gut health	
Processed meats	Sausages, deli meats	Cell wall fragments, SCFAs	Improved nutrient profile, antimicrobial action, and immune modulation	

Table summarizes how postbiotics are applied across various processed food categories, directly complementing Section 3.3.3 by linking practical examples to mechanisms and benefits. It bridges theory and application, showing how postbiotics enhance health, flavor, and shelf life in products like dairy, baked goods, and beverages

As described in Fig. 9, gut-derived postbiotic metabolites, primarily SCFAs, indole derivatives, and peptides, interact systemically with key organs involved in metabolic and immune regulation. These postbiotics engage intestinal receptors such as GPR41, GPR43, GPR4, TLRs, and NOD-like receptors, modulating downstream signaling pathways that extend beyond the gut. In the liver, they inhibit lipogenesis and enhance insulin sensitivity, while in adipose tissue, they suppress hypertrophy and promote the production of anti-inflammatory cytokines[25,26]. In the pancreas, they boost insulin secretion and β-cell preservation[25,26]. Postbiotics also promote immune regulation by increasing regulatory T cells and reducing IL-6 and TNF-α[26]. Additionally, via the gut–brain axis, they influence vagal signaling to modulate satiety and stress responses[26].

Applications in different processed food categories: Postbiotics are increasingly incorporated into processed food products due to their health benefits, which include gut microbiota modulation, immune enhancement, and metabolic improvement. Their integration spans dairy, beverages, bakery products, and snacks[27].

Table 3 showcases the inclusion of SCFAs, peptides, and heat-killed LAB in processed food categories such as dairy, beverages, and snacks. These compounds support gut function, immune activity, and metabolic balance. Functional applications in yogurt, kombucha, and enriched bread emphasize their health potential and preventive roles against chronic diseases. Their incorporation also enhances product quality, shelf stability, and consumer appeal[27,28].

As illustrated in Fig. 10, the infographic outlines the integration of postbiotics into five major categories of processed foods: Dairy, functional beverages, bakery products, snacks, and processed meats. Each category features distinct postbiotic components such as SCFAs and peptides linked to specific physiological benefits. Dairy products and functional beverages primarily promote gut barrier integrity and immune modulation through SCFA activity[27,28]. Bakery items and snacks, often enriched with peptides and fermentation metabolites, contribute to gut microbiota balance, anti-obesity effects, and improved glycemic control[27,28]. Although less conventional, processed meats also serve as effective carriers of postbiotics, offering potential antimicrobial and nutritional enhancements[27,28].

In fermented dairy products, postbiotics derived from Lactobacillus and Bifidobacterium strains demonstrate beneficial effects such as gut microbiota modulation, improved barrier function, and inflammation reduction. Heat-killed Lactobacillus in yogurt, for example, has been shown to influence microbial composition and enhance gut health[27,28].

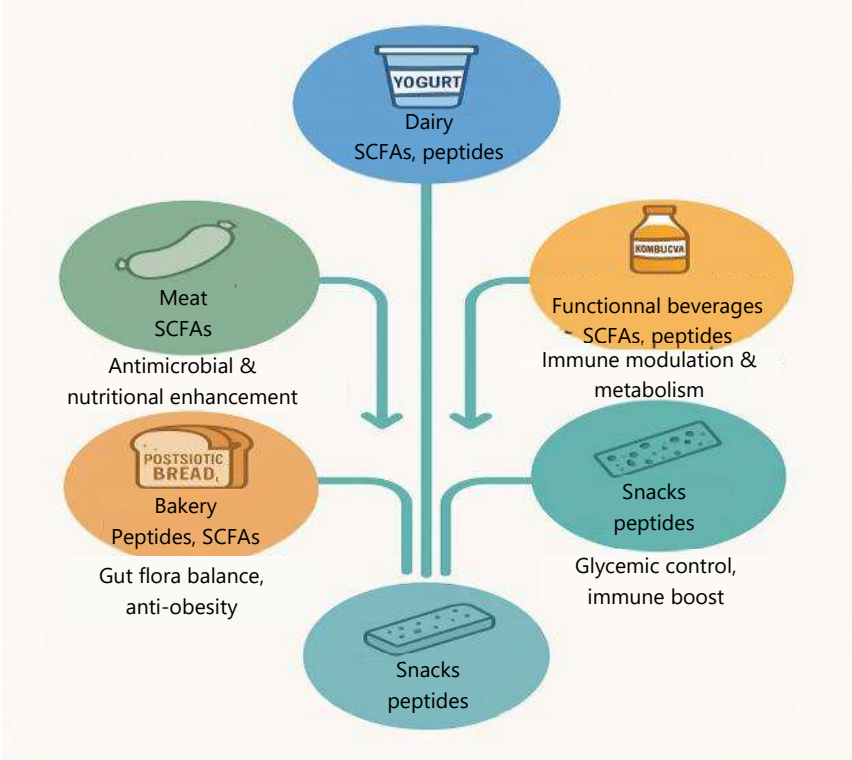

Fig. 10: Postbiotic applications across processed food categories (self-generated)

This diagram illustrates the incorporation of postbiotics; mainly short-chain fatty acids (SCFAs) and peptides into various processed food categories such as dairy, functional beverages, bakery products, snacks, and meats. It highlights the associated health benefits, including immune modulation, gut flora balance, glycemic control, anti-obesity effects, and antimicrobial activity. Each category demonstrates how specific postbiotic components contribute to physiological health outcomes

Functional beverages like kombucha and kefir are valued for both probiotic and postbiotic effects. These drinks contain bioactives such as SCFAs that support gut integrity, immune function, and metabolic regulation[27,28].

In bakery products, postbiotics elevate the health value of conventional foods. Postbiotic-enriched bread offers prebiotic-like effects by promoting beneficial microbiota, while fermentation contributes bioactive peptides and SCFAs for gut and metabolic health[28].

Snacks and processed meats are gaining popularity as postbiotic delivery formats. Functional snacks support immunity, glycemic control, and gut health[28]. Fortified processed meats enhance the nutritional profile without requiring live bacteria[28].

These diverse applications showcase postbiotics functional versatility and consumer appeal in modern processed food systems.

Bioavailability and future research directions: The bioavailability of postbiotics is a critical determinant of their effectiveness in delivering health benefits. It refers to the extent and rate at which these compounds are absorbed and become available at the site of physiological action. Several factors influence postbiotic bioavailability, including the nature of the compound, the food matrix in which it is delivered, and gastrointestinal conditions such as pH, enzymatic activity, and the presence of bile salts[29].

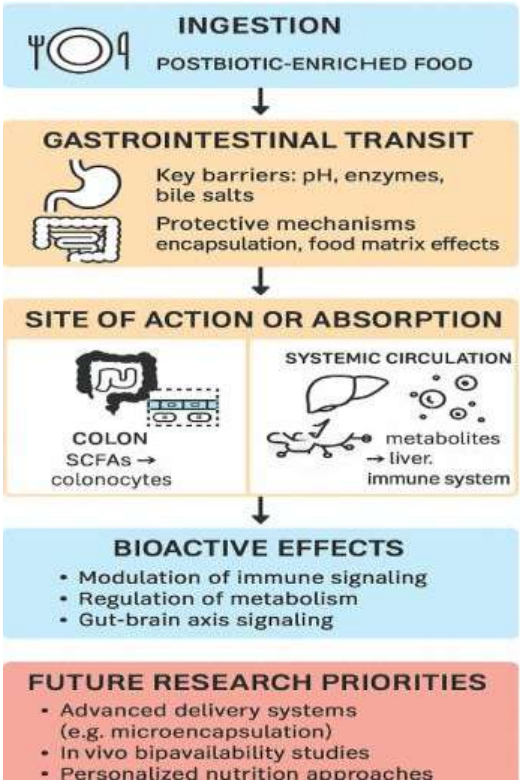

Fig. 11: Postbiotic bioavailability and action path in the host (self-generated)

This diagram illustrates the journey of postbiotics from ingestion through the gastrointestinal tract to their sites of action in the colon and systemic circulation. It highlights key influencing factors such as pH, enzymes, and encapsulation methods that affect bioavailability. The figure also outlines physiological effects on liver function, immune signaling, metabolism, and the gut–brain axis

As illustrated in Fig. 11, postbiotics follow a complex journey from ingestion to systemic effect. Upon consumption via postbiotic-enriched foods, these compounds navigate the gastrointestinal tract where they encounter environmental variables that affect their stability and absorption[29]. To enhance stability, encapsulation techniques and tailored matrix formulations are often employed[29,30].

Once they reach their primary sites of action typically the colon or systemic circulation, Short-Chain Fatty Acids (SCFAs) and other metabolites interact with colonocytes or enter the bloodstream. There, they influence various physiological processes including liver function, immune signaling, and metabolic regulation[29,30]. In addition, these postbiotic actions extend to the gut–brain axis through neuroimmune communication pathways[29,30].

The bottom panel of Fig. 11 highlights key research priorities aimed at advancing the field of postbiotics. These include the development of innovative delivery systems, *in vivo* testing to assess bioavailability, and clinical studies to validate health outcomes[30]. Emerging areas of exploration involve personalized nutrition approaches and deeper investigations into the interactions between postbiotics and the gut–brain axis, immune responses, and metabolic pathways[30]. Progress in this field will also depend on the application of advanced analytical techniques, such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS), for precise quantification of bioactive compounds in complex food matrices[30].

Postbiotics in functional foods: Roadmap for future innovation and clinical application: Postbiotics, nonliving microbial metabolites with proven biological activity, are increasingly recognized as promising components in the formulation of functional foods. Unlike probiotics, postbiotics do not rely on the viability of microbial cells to exert health benefits, making them more stable and suitable for various food processing conditions. Their demonstrated roles in supporting gastrointestinal health, enhancing immune function, and regulating metabolic processes underscore their potential in promoting overall wellness[31].

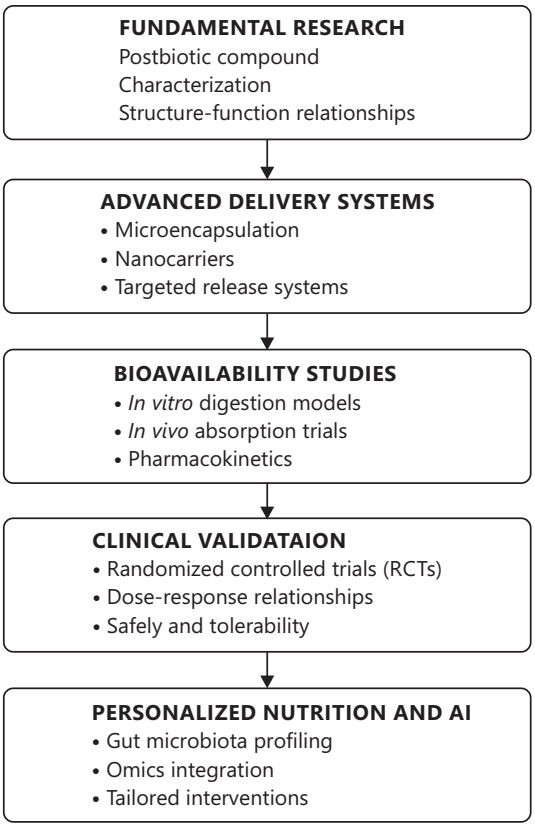

Fig. 12: Future research roadmap for postbiotic innovation (self-generated)

This diagram outlines the progressive phases in postbiotic research and application, from fundamental compound characterization to personalized nutrition. Each step, including delivery systems, bioavailability studies, clinical validation, and AI integration, supports the translation of postbiotics into effective functional foods. The framework emphasizes precision, safety, and targeted health outcomes

As illustrated in Fig. 12, the future of postbiotic science is envisioned through a structured and integrative research roadmap. The first phase, Fundamental Research, focuses on the identification and characterization of postbiotic compounds and the elucidation of structure-function relationships, guided by advanced omics platforms and microbial metabolomics[31].

The second phase involves the development of Advanced Delivery Systems, such as microencapsulation and nanocarrier technologies, which are designed to enhance the stability and targeted delivery of postbiotics within diverse food matrices. This is followed by Bioavailability Studies, employing both *in vitro* gastrointestinal simulation models (e.g., SHIME, TIM-1) and *in vivo* animal and human trials to evaluate absorption dynamics and pharmacokinetic behavior[31].

Next is the clinical validation phase, where Randomized Controlled Trials (RCTs) play a crucial role in determining safety, tolerability, and effective dosing regimens across different population groups.

The final phase emphasizes the integration of Personalized Nutrition and Artificial Intelligence, enabling precision dietary strategies through individualized microbiota profiling and multiomics data analysis. This advancement positions postbiotics at the forefront of digital health innovation and targeted nutritional therapy.

Moving forward, dedicated research will be critical to unlocking the full therapeutic and nutritional potential of postbiotics. Deeper insights into their bioavailability, efficacy, and long-term health effects are essential. Clinical trials will be instrumental in formulating evidence-based recommendations, while technological advances in food processing will facilitate more efficient and consumer-friendly delivery systems.

As science and technology continue to evolve, postbiotics are well-positioned to shape the next generation of functional foods, products that not only fulfill nutritional requirements but also contribute meaningfully to the prevention and management of chronic diseases through personalized, scientifically informed strategies.

CONCLUSION

Postbiotics represent a promising class of bioactive compounds with significant potential in advancing functional food science and human health. Their well-documented roles in modulating immunity, enhancing intestinal barrier function, and supporting metabolic regulation underscore their therapeutic relevance. This review highlights the importance of biochemical characterization, processing stability, and precise delivery systems for maximizing postbiotic efficacy. Integrating postbiotics into diverse food matrices offers a practical route to achieving targeted nutritional benefits. Continued research, including clinical validation and personalized nutrition strategies, will be key to unlocking their full potential. Postbiotics stand at the intersection of biochemistry, food technology, and preventive health care.

SIGNIFICANCE STATEMENT

This manuscript presents a comprehensive synthesis of current knowledge on postbiotics, emphasizing their biochemical mechanisms, stability in processed foods, and health-promoting effects. By integrating insights from microbiology, nutritional biochemistry, and food technology, it highlights how postbiotic compounds such as short-chain fatty acids, peptides, and microbial cell wall fragments interact with host systems to enhance gut integrity, modulate immune responses, and support metabolic regulation. The work also addresses critical challenges in maintaining postbiotic functionality during food processing and storage, offering practical strategies and analytical approaches to ensure efficacy. By bridging fundamental science and applied nutrition, this study provides a valuable framework for the development of next-generation functional foods with targeted health benefits.

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