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Review article

FOOD-DERIVED GARLIC POLYSACCHARIDES AS EMERGING FUNCTIONAL INGREDIENTS: STRUCTURE, MICROBIOTA–IMMUNITY INTERACTIONS, AND HEALTH IMPLICATIONS

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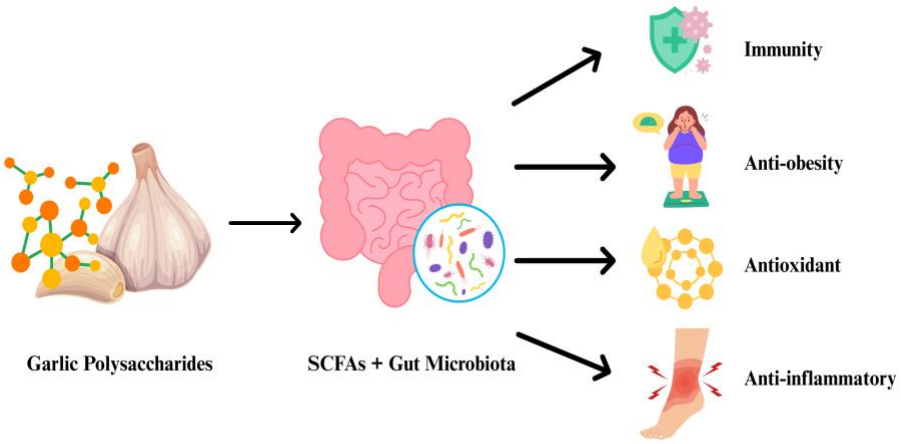
Abstract. Garlic polysaccharides (GPs) are emerging as important non-sulfur bioactives that complement the well-studied organosulfur compounds of Allium sativum. With diverse structures and molecular weights, GPs exert antioxidant, anti-inflammatory, immunomodulatory, and metabolic benefits that are increasingly linked to their role as prebiotic substrates for gut microbiota. This review consolidates recent advances in the extraction and structural characterization of GPs and examines how their physicochemical features shape fermentability, microbial enrichment, and production of metabolites such as short-chain fatty acids, bile acids, and tryptophan derivatives. These microbiota-derived signals, together with direct immune modulation by specific GP fractions, underpin improvements in mucosal barrier function, systemic immunity, and metabolic outcomes in preclinical models of obesity, diabetes, fatty liver disease, and atherosclerosis. By integrating structure–function relationships with microbiota–immunity interactions, we outline the dual role of GPs as prebiotics and immunonutrients, and compare their actions with those of established dietary polysaccharides. Current limitations include methodological variability, lack of standardized structural reporting, and scarce clinical validation. Future directions call for multi-omics approaches, personalized nutrition strategies, and well-designed human trials to translate the promising microbiota–immune mechanisms of GPs into functional food and therapeutic applications.



Graphical Abstract

1.Introduction

Garlic (*Allium sativum L.)* has a significant history as a culinary ingredient and a folk remedy, attributed with extensive health effects, including cardiometabolic protection and immune modulation, among others. Modern studies have long focused on organosulfur-based compounds, but there is growing interest in the role of carbohydrate fractions in determining the functional profile of garlic, namely polysaccharides and oligosaccharides. According to recent narrative and systematic literature, garlic-derived polysaccharides (GPs) have a wide range of bioactivities and should be given special emphasis in food and health studies (El-Saadony *et al*., 2024).

Among the non-sulfur constituents, GPs are structurally heterogeneous: most edible-bulb fractions are fructan/oligofructose-like (inulin-type) with β- (2-1)-linkages and isolated branching, though by-product streams (peels, leaves, pomace) may be pectin-rich, having galacturonic acid and rhamnogalacturonan-I/homogalacturonan domains. Since 2018, progress has mapped extraction-structure relationships (hot water/enzymatic routes; ultrafiltration; chromatographic fractionation) and has related molecular weight/branching to functional performance (Jiang *et al*., 2022; Qiu *et al*., 2024; Sunanta *et al*., 2024).

Concurrently, the gut microbiota has emerged as a central mediator of diet–health relationships. In vitro and in vivo studies show garlic saccharide fractions act as prebiotic substrates, selectively enriching beneficial taxa (e.g., *Bifidobacterium*, *Akkermansia*), enhancing short-chain fatty acid (SCFA) production, and improving barrier and inflammatory readouts; human evidence is still limited but growing. Notably, water-soluble garlic polysaccharides (WSGP) alleviate colitis and restore mucosal integrity in murine models, and aged/processed garlic saccharides can remodel microbial communities alongside favorable metabolic markers—supporting a microbiota-linked mode of action (Ettehad-Marvasti *et al*., 2022; Ha *et al*., 2024; T. Li *et al*., 2024a; Shao *et al*., 2024; Zhao *et al*., 2022a).

This review synthesizes the structural features, biological activities, and gut-microbiota interactions of garlic polysaccharides/oligosaccharides, emphasizing a structure–function–activity lens rather than a mere catalogue of studies. We integrate recent advances (2018–2025) across extraction/characterization, prebiotic and immunomodulatory outcomes, and microbiota-linked health implications, highlighting where structure (degree of polymerization, branching, and pectin vs. fructan signatures) plausibly shapes fermentability and host responses. Our approach is narrative and integrative, drawing together chemistry, microbiology, and nutrition

to clarify current evidence, limitations, and opportunities for translational research and functional food development (Holmes *et al*., 2022; Qiu *et al*., 2024).

2.Structural Features of Garlic Polysaccharides

2.1. Extraction and purification methods

Garlic polysaccharides (GPs) are most commonly obtained by hot-water extraction of fresh or dried garlic tissues, followed by removal of low-molecular impurities and concentration, with ethanol precipitation used to recover crude polysaccharide fractions. Subsequent deproteinization (Sevag or enzymatic), dialysis or ultrafiltration, and chromatographic fractionation (ion-exchange and size-exclusion) produce purified fractions of defined molecular weight ranges. Ultrasonic/microwave-assisted extraction and aqueous two-phase systems have also been applied to improve yields and reduce extraction time, while enzymatic-assisted methods enable milder conditions that better preserve native structures. Gradient ethanol precipitation and membrane separation (ultrafiltration) are widely used to obtain oligosaccharide vs. higher molecular-weight polysaccharide fractions for downstream characterization and bioactivity testing (M. Wang & Cheong, 2023; Y. Zhang *et al*., 2024).

Choice of extraction/purification method strongly affects yield, degree of polymerization (DP), and apparent bioactivity — for example, hydrolytic or harsh chemical methods can shorten chain length and increase fermentability, whereas gentle aqueous extraction preserves higher-MW fractions. Recent method comparisons and optimizations (including response-surface and design-of-experiments) have been reported to balance yield and structural integrity (Zhi *et al*., 2023).

Table 1. Extraction methods and structural features of garlic polysaccharides.

Extraction / Purification Method, Yield (if reported), and Structural Features	Analytical Techniques Used	References
Hot-water extraction (60 °C, 180 min, 1:10 w/v) followed by ethanol precipitation, deproteinization and dialysis. Yield not specified. Polysaccharide fraction rich in fructose (82.8%) and glucose (16.8%), Mw ≈3.7 kDa; inulin-type β-Fruf linkages.	HPLC for monosaccharides, SEC for Mw, FTIR, UV/CD spectra, SEM, thermal analysis.	Preparation & characterization of garlic polysaccharides (Bai *et al*., 2022)
Acidolysis of crude GPs + ultrafiltration to obtain low- and high-Mw fractions (e.g., U0.3, U6). Yield not expressed. Fractions varied in DP and fermentability; fructose and glucose were dominant.	HPAEC/HPLC, HPSEC, simulated digestion/fermentation, 16S rRNA sequencing.	Digestive properties and prebiotic activity of garlic saccharides (Zhao *et al*., 2022a)
Three-phase partitioning + gradient ethanol fractionation. Yield fraction-dependent. Produced multiple Mw populations with distinct monosaccharide ratios; separated oligosaccharides from polysaccharides.	SEC-MALLS/HPSEC, monosaccharide analysis, FTIR, GC–MS linkage analysis.	Three-phase partitioning + gradient ethanol fractionation (example of advanced fractionation used for

		raw garlic) (Yan *et al*., 2021)
Sequential acidic extraction and fractionation of garlic biomass. Reported higher yields in pectin-rich fractions. Structures dominated by galacturonic acid (>61%), with Gal and Rha; homogalacturonan and RG-I domains; Mw ≈350 kDa; degree of methylation 44–56%.	HPLC, methylation/GC–MS, FTIR, viscometry, Mw profiling.	Fractionation & characterisation of pectin-rich extracts from garlic biomass (Sunanta *et al*., 2023)
Hot-water extraction followed by purification (based on prior protocols) was used to obtain water-soluble GP fraction (WSGP). Yield not reported. Composition consistent with fructan-rich, fermentable oligosaccharides; bioactive in colitis model.	Monosaccharide analysis, LC–MS metabolites, WB/IF for bioactivity.	Water-soluble garlic polysaccharide (WSGP) improves ulcerative colitis (Shao *et al*., 2024a)
Extraction/fractionation of aged garlic produced aged garlic oligosaccharides (AGOs). Yield details not main focus. Fractions were low-DP oligosaccharides increasing SCFAs and reducing TMAO; increased Akkermansia reported.	SEC/HPSEC, monosaccharide analysis, GC/LC–MS for SCFAs, 16S/metagenomics.	Aged garlic oligosaccharides (AGOs) prepared from aged garlic (X. Wang *et al*., 2025)

2.2. Structural characteristics

Garlic polysaccharide fractions are compositionally diverse. Fructan-type fractions (common in garlic bulbs) are dominated by fructose (with terminal glucose residues in some chains), while other garlic-derived fractions—especially from peels, leaves or processing residues—can be pectin-rich, containing high proportions of galacturonic acid plus neutral sugars such as galactose, arabinose, glucose, xylose and rhamnose. Reported monosaccharide profiles in recent studies list combinations of glucose, fructose, galactose, arabinose, mannose and xylose depending on tissue source and extraction method (Chen *et al*., 2024; Qiu *et al*., 2024; Sunanta *et al*., 2024).

The predominant backbone in bulb-derived garlic saccharides is inulin-type fructan built from β-(2→1) fructofuranosyl linkages; occasional β-(2→6) branching is reported in some preparations. Pectin-type fractions contain homogalacturonan (HG) and rhamnogalacturonan-I (RG-I) regions with side chains of arabinans/galactans. Degree of polymerization (DP) ranges from short chain fructooligosaccharides (DP < 10) to long inulins (DP up to several tens), and measured molecular weights (Mw) for garlic polysaccharide fractions span from a few kDa (oligosaccharides) to high-MW polysaccharides (>100 kDa), depending on fractionation and analytical method. Linkage analysis (methylation/GCMS) and NMR studies confirm the β-(2→1) fructan motif in many bulb fractions, and pectic linkages in peel/leaf fractions (Karimi *et al*., 2025; Qi *et al*., 2022).

Operationally, garlic oligosaccharides generally refer to low-DP, water-soluble

fructooligosaccharides (FOS) and short fructans that are highly fermentable by gut microbes; polysaccharides indicate broader, higher-Mw fractions including long-chain fructans and pectin-type polymers with different physicochemical behaviors. Fractionation (e.g., graded ethanol precipitation, ultrafiltration, or chromatographic collection) is typically used to produce and distinguish these classes for functional testing (Ito *et al*., 2011; M. Li *et al*., 2023). Fig. 1 shows the schematic structure of garlic polysaccharides.

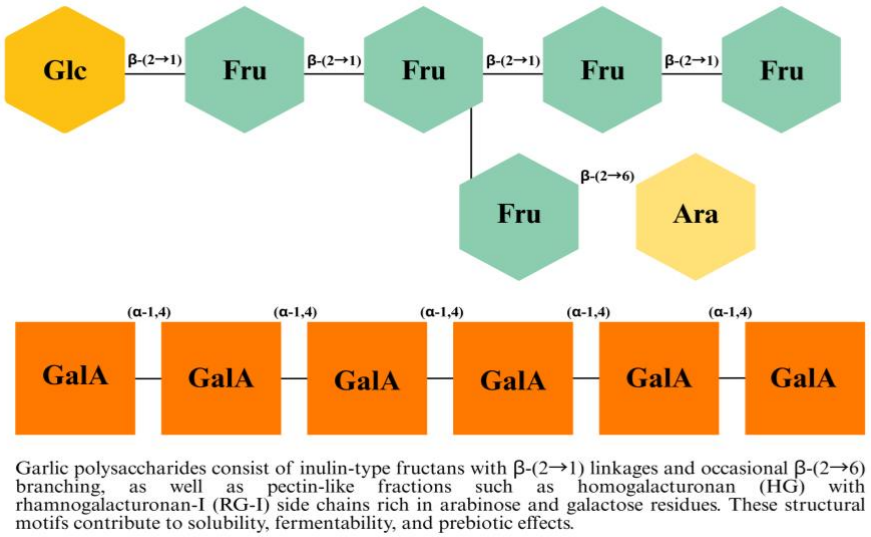


Figure 1. Schematic structure of garlic polysaccharides

2.3. Structure-Function Relationship

Water solubility is a primary determinant of microbial accessibility and fermentability. Low-DP, water-soluble oligosaccharides are rapidly fermented by saccharolytic gut bacteria resulting in quick SCFA production, whereas high-Mw, less soluble polymers may be more slowly fermented or partially resistant, stimulating different microbial consortia. Thus, solubility and molecular size together shape fermentation kinetics and selective enrichment of taxa (D. T. Wu *et al*., 2022; Xia *et al*., 2025).

Multiple recent studies across plant polysaccharides show that lower Mw/DP fractions tend to be fermented more readily and promote greater increases in SCFAs (acetate, propionate, butyrate) and beneficial microbes (e.g., *Bifidobacterium*, *Lachnospiraceae*), whereas higher-Mw fractions produce slower but sometimes more sustained effects; these patterns have been observed for fructans and pectic-oligosaccharides alike and are reported for garlic fractions as well. Experimental work demonstrates that enzymatic hydrolysis or acidolysis to reduce DP can increase prebiotic potency (M. Li *et al*., 2023; Xia *et al*., 2025; Xiao *et al*., 2025).

Branching can influence enzyme accessibility and the spectrum of microbes able to degrade the polymer: more highly branched polysaccharides may favor specialized taxa possessing debranching enzymes, while linear β-(2→1) fructans are broadly fermented by common saccharolytic bacteria. Likewise, the presence of uronic acids (as in pectic regions) introduces charged groups that affect solubility and interactions with host mucins, and thus may modulate colon localization and immunomodulatory potential (Qi *et al*., 2022; D. T. Wu *et al*., 2022).

Structural features also correlate with reported biological effects beyond prebiotic fermentation: low-Mw garlic oligosaccharides are often linked to stronger in vitro prebiotic effects and SCFA increases that relate to metabolic endpoints (glycemic control, lipid modulation), while certain pectin-rich fractions display viscosity/emulsifying properties and antioxidant capacities that may contribute to gut barrier protection and anti-inflammatory effects.

However, direct structure–activity causal links remain understudied in human trials and require standardized fractionation and comparative head-to-head experiments (Chen *et al*., 2024; Qiu *et al*., 2024; M. Wang & Cheong, 2023).

3. Biological Activities of Garlic Polysaccharides

3.1. Antioxidant activity

Garlic polysaccharides (GPs) consistently show free-radical scavenging (e.g., DPPH, ABTS, •OH) and reducing power in vitro, with activity influenced by molecular weight and chemical modification. Carboxymethylated or metal-complexed derivatives generally enhance total antioxidant capacity compared with native GP, suggesting that electron-donating substituents and coordination with metal ions improve redox performance (Bai *et al*., 2022; Cheng *et al*., 2020). Recent in vivo work also reports improved antioxidant enzyme activities (SOD, CAT, GSH-Px) following GP supplementation, alongside mitochondrial energy benefits via AMPK/PGC-1α signaling in mice subjected to exhaustive exercise, supporting functional relevance beyond test-tube assays (T. Li *et al*., 2024b).

Table 2. Biological activities of garlic polysaccharides.

Study	Model / GP fraction & dose (as reported)	Key outcomes (bioactivity)
(Shao *et al*., 2024b)	DSS-induced colitis mice; WSGP (water-soluble garlic polysaccharide); in vivo dosing 200–400 mg/kg (oral).	Attenuated colitis: ↓histological damage, ↑tight-junction proteins (ZO-1, occludin), ↓pro-inflammatory cytokines (TNF-α, IL-6), modulated NF-κB/STAT3 signaling; increased fecal SCFAs.
(X. Wang *et al*., 2025)	ApoE$^{-/-}$ mice on HFD/HCD; Aged garlic oligosaccharides (AGOs) isolated from aged garlic; fraction doses per Methods.	Reduced atherosclerotic lesion formation, ↓TMA/TMAO, ↑fecal SCFAs (acetate/propionate/butyrate), remodeled microbiota (↑Akkermansia), improved lipid profile.
(Wu *et al*., 2024)	In vitro RAW264.7 macrophage cells & immunosuppressed mice; fructan-type garlic polysaccharide (purified fraction).	Immunostimulatory under immunosuppression: ↑NO, ↑TNF-α, ↑IL-6 in macrophages; restored immune indices in immunosuppressed mice (enhanced macrophage function).

(Liu *et al*., 2024)	HFD-induced obese mice; fermented garlic polysaccharides (BGP/OPS); dosing per study methods.	Anti-obesity & hypolipidemic effects: ↓body weight gain, ↓serum TG/TC, improved adipose inflammation; associated with altered gut microbiota and increased fecal SCFAs.
(T. Li *et al*., 2024)	Mouse fatigue model; soluble garlic polysaccharide from industrial garlic waste; dosing per Methods.	Improved exercise endurance, ↑hepatic & muscle glycogen, ↑antioxidant enzymes (SOD, GSH-Px, CAT), activated AMPK/PGC-1α signaling—showing antioxidant and metabolic benefits.
(Xie *et al*., 2022)	Animal metabolic models; garlic polysaccharide fractions (reported).	Demonstrated hypolipidemic and metabolic benefits; proposed GP as a nutraceutical for metabolic syndrome/T2D models.
(Cheng *et al*., 2020)	In vitro antioxidant assays & fraction testing (P, SP, PP, CMP) using garlic raw material.	Demonstrated significant DPPH/ABTS/•OH scavenging and reducing power for garlic polysaccharide fractions; chemical modification (e.g., carboxymethylation) enhanced antioxidant capacity.

3.2. Anti-inflammatory activity

Water-soluble garlic polysaccharides (WSGP) attenuate experimental colitis by dampening NF-κB/STAT3 signaling, reducing pro-inflammatory cytokines (e.g., TNF-α, IL-6), and enhancing mucosal barrier integrity (tight-junction proteins), with concurrent modulation of gut microbial metabolites. These findings have been reproduced in multiple DSS colitis models and updated analyses (2020–2024), highlighting a robust anti-inflammatory profile (Shao *et al*., 2020, 2024). Mechanistically, lower-MW ("small molecular") GP fractions often show stronger anti-inflammatory effects—consistent with improved solubility/fermentability—though authors call for standardized, head-to-head comparisons across fractions (M. Lu *et al*., 2023) .

3.3. Immunomodulatory activity

Beyond generic anti-inflammation, specific GP fractions activate innate immune cells. A 2024 study on a fructan-type GP demonstrated macrophage (RAW264.7) activation (↑NO, TNF-α, IL-6) and immune enhancement in immunosuppressed mice, linking activity to fructan structure (J. Wu *et al*., 2024). Broader reviews on plant polysaccharides corroborate that structural motifs and branching patterns shape macrophage polarization and downstream cytokine milieus, positioning GPs as candidate

immunonutrients; however, direct clinical validation for garlic-specific fractions remains limited (Wei *et al*., 2024).

3.4. Metabolic Health Benefits

Evidence is accumulating that GPs improve metabolic phenotypes, often in concert with microbiota remodeling. Polysaccharides from fermented garlic reduced weight gain and improved lipid profiles in HFD mice, with shifts in gut microbiota and faecal SCFAs indicating a prebiotic mechanism (Q. Liu *et al*., 2024). Purified GP improved glycemic control and hepatic glycogen metabolism in T2DM models, complementing broader evidence that dietary polysaccharides modulate glucose homeostasis (He *et al*., 2023; Xie *et al*., 2023). Aged garlic oligosaccharides mitigated atherosclerosis in ApoE$^{-/-}$ mice fed a high-fat/high-cholesterol diet, accompanying microbiota and metabolic improvements; separate studies report GP-mediated protection in MAFLD models (J. Liu *et al*., 2022; X. Wang *et al*., 2025).

4. Garlic Polysaccharides and Gut Microbiota

4.1. Prebiotic effects

Garlic polysaccharides (GPs), particularly low-molecular-weight fructan/oligosaccharide fractions obtained by controlled hydrolysis or graded fractionation, display clear **prebiotic activity** in vitro and in vivo. Several studies demonstrate that GPs (or garlic saccharide fractions) are selectively fermented by beneficial gut microbes, promoting the growth of genera commonly associated with health, such as *Bifidobacterium* and *Lactobacillus*. In vitro fermentation assays using human faecal inocula showed that hydrolysed garlic saccharides increased bifidogenic activity and produced greater levels of short-chain fatty acids (SCFAs) compared with native, high-DP fractions — consistent with the established relation between degree of polymerization and fermentability (X. Lu *et al*., 2021; Zhao *et al*., 2022).

Animal studies corroborate these findings: dietary supplementation with garlic oligosaccharide or water-soluble GP fractions increased relative abundances of *Bifidobacterium*, *Lactobacillus*, and other saccharolytic taxa, while reducing opportunistic/pathobiont groups in rodent models. These microbial shifts were accompanied by rises in faecal acetate, propionate, and butyrate — metabolites that mediate many downstream physiological effects, including epithelial energy supply, barrier integrity, and immune signalling. Such prebiotic responses appear more pronounced for low-DP/low-Mw fractions and for processed/aged garlic oligosaccharides versus unprocessed high-Mw polysaccharides (X. Wang *et al*., 2025; Zhao *et al*., 2022b). Lu *et al*. (2021) characterized garlic neutral polysaccharides and showed enhanced in vitro fermentation and bifidogenic effects after controlled hydrolysis. A series of more recent in vivo reports (2022–2024) confirm that water-soluble and aged garlic saccharides enrich beneficial taxa and increase fecal SCFAs in animal models (T. Li *et al*., 2024a; X. Lu *et al*., 2021).

Table 3. Comparative features of garlic vs. other polysaccharides.

Feature	Garlic polysaccharides (GPs)	Inulin / Fructooligosaccharides (FOS)	β-Glucans
Source & primary composition	Mainly fructan-type (inulin-like) oligo-/polysaccharides in bulbs (β-(2→1) Fruf	Fructans composed largely of β-(2→1) fructofuranosyl linkages (inulin) or shorter DP (FOS); well-	β-(1→3)/(1→6)-linked glucose polymers from yeast, fungi, oats, barley; structure

	backbone; occasional β-(2→6) branches); pectin-rich fractions (GalA, RG-I) often found in peels/leaves/waste (Jiang *et al*., 2022).	characterized commercial prebiotics (Hughes *et al*., 2022)	(branching/conformation) varies with source. Strongly associated with immunomodulatory activity (Zhong *et al*., 2023).
Typical molecular size / DP	Broad: GP range from low-DP oligosaccharides (few kDa) to high-Mw polysaccharides (tens to hundreds kDa), depending on extraction/fractionation. DP influences fermentability (Zhao *et al*., 2022a).	Commercial inulin DP typically 2–60; FOS DP <10 (short-chain), consistent and reproducible manufacturing profiles. DP correlates with fermentation kinetics (Hughes *et al*., 2022).	Mw and degree of branching highly source-dependent (oligomeric to >100 kDa); triple-helix vs single chain conformations influence receptor binding and activity (Zhong *et al*., 2023).
Primary biological actions	Prebiotic, antioxidant, immunomodulatory (macrophage activation, cytokine modulation), anti-inflammatory, metabolic benefits in animal models (anti-obesity, hepatoprotection) (Shao *et al*., 2024a).	Prebiotic effects (bifidogenic), improved laxation, enhanced mineral absorption, metabolic benefits (improved insulin sensitivity, reduced TGs) in human and animal studies (J. Li *et al*., 2025).	Immunomodulation via pattern recognition receptors (Dectin-1, CR3, TLR cross-talk); stimulates macrophages, dendritic cells and NK cells; also acts as a fermentable fiber in some sources (partial prebiotic effects) (Singh & Bhardwaj, 2023).
Prebiotic potency & taxa stimulated	Promotes *Bifidobacterium*, *Lactobacillus* and some SCFA-producers (e.g.,	Robust bifidogenic effect in humans and animals; reliably increases SCFAs (acetate/propionate/butyrate)	Variable: some β-glucans (e.g., oat β-glucan) increase SCFAs and beneficial taxa, but effects are source- and

	Lachnospiraceae); potency depends on DP and fraction (low-DP often more fermentable). Evidence primarily in vitro and rodent models; some human-relevant fermentation shown in vitro (Zhao *et al*., 2022a).	and beneficial taxa in many RCTs and reviews (Holmes *et al*., 2022).	solubility-dependent; yeast/fungal β-glucans more prominent for direct immune effects (Feng *et al*., 2025).
Mechanisms linking microbiota to immunity	Indirect: promote fermentation leading to SCFAs (GPR41/43, HDAC inhibition) and likely effects on bile acids/trp-metabolites that support Treg induction and barrier protection; Direct: certain GP fractions can stimulate innate immune cells (macrophages) in vitro. Evidence largely preclinical (Y. Zhang *et al*., 2024).	Indirectly via SCFA production, increased mucosal health, and lowered endotoxemia; substantial human mechanistic evidence for SCFA-driven effects on metabolism and some immune endpoints (Holmes *et al*., 2022).	Direct receptor-mediated immune activation (Dectin-1, others) leading to cytokine modulation, adaptive responses; Indirect microbiota-metabolite effects also reported for some dietary β-glucans. Strong mechanistic evidence from in vitro, animal studies and some clinical trials (Zhong *et al*., 2023).
Clinical evidence	Limited direct RCTs with isolated GP fractions; most evidence from in vitro and animal models,	Multiple human RCTs and meta-analyses supporting tolerance, bifidogenic response, improvements in bowel habits and some	Several human studies and trials (mostly for yeast/beta-glucan supplements and oat β-glucan for cholesterol) show immune or metabolic

	with a few human studies using whole/processed garlic or GP supplements showing microbiota/modulatory signals — calls for well-designed human trials (T. Li *et al*., 2024).	metabolic markers; inulin/FOS are clinically established prebiotics (J. Li *et al*., 2025).	benefits; more trials exist for β-glucan than for GPs, though specifics depend on source and formulation (Muroya *et al*., 2025).
Unique advantages / translational potential	Dual role: acts as a prebiotic and contains fractions with direct immunomodulatory activity — promising for metabolic diseases via flora-metabolites-immune axis; also potential to use agricultural waste (peels) as pectin sources. However, heterogeneity and lack of human RCTs limit current translation (Zhao *et al*., 2022a).	Well-characterized, consistent (commercial) source; predictable fermentation; widely used as benchmark prebiotics and included in many functional foods; regulatory acceptance for many uses (Hughes *et al*., 2022).	Strong evidence for immune activation and some metabolic endpoints (cholesterol reduction for oat β-glucan); established as immunonutrients in clinical nutrition and as functional ingredients. Source variability requires careful standardization (Singh & Bhardwaj, 2023).

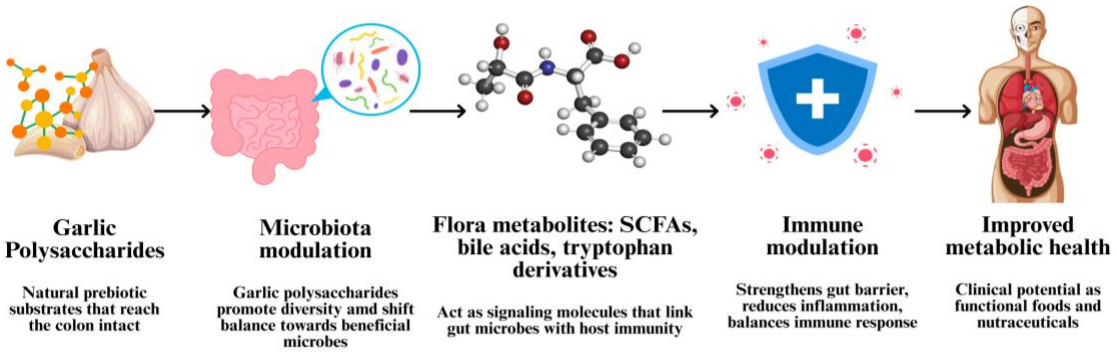


Figure 2. Mechanism of garlic polysaccharides in modulating gut microbiota and their metabolites

4.2. Impact on microbial diversity and balance

Beyond selective stimulation, GPs can reshape overall gut community structure and improve dysbiosis in disease models. In DSS-induced colitis and other inflammatory rodent models, supplementation with water-soluble garlic polysaccharides restored alpha diversity (Shannon/Chao indices) and partially reversed disease-associated shifts such as Firmicutes/Bacteroidetes imbalance. These community-level changes frequently co-occur with reduced pro-inflammatory taxa and increased mucin-degrading or SCFA-producing lineages (e.g., *Akkermansia*, Lachnospiraceae members), suggesting that GPs can promote a more resilient, functionally favorable microbiome (Shao *et al*., 2024).

In metabolic disease contexts (high-fat diet or ApoE$^{-/-}$ atherosclerosis models), aged garlic oligosaccharides and other garlic saccharide supplements have been reported to lower the Firmicutes: Bacteroidetes ratio, increase abundances of SCFA-producers, and reduce microbial signatures linked to TMA/TMAO production. These compositional shifts parallel improvements in body weight, lipid profiles, and inflammatory markers, supporting a microbiota-mediated route for garlic polysaccharide benefits in metabolic disorders (Ha *et al*., 2024; X. Wang *et al*., 2025). Most robust data come from animal and in vitro studies; human interventional data specifically with isolated garlic polysaccharide fractions remain scarce. Heterogeneity in extraction/fractionation methods, dosing regimens, and sequencing/analysis pipelines also complicates direct comparisons across studies. Nevertheless, the converging evidence supports the concept that garlic polysaccharides—especially low-DP fractions—act as meaningful prebiotic modulators that improve microbial diversity and functional outputs in models of intestinal inflammation and metabolic perturbation (X. Lu *et al*., 2021; Vinelli *et al*., 2022).

4.3. Flora Metabolites as Key Mediators

Dietary polysaccharides (including garlic-derived oligo-/polysaccharides) primarily exert systemic effects by being fermented or transformed by the gut microbiota into small bioactive molecules. The three categories most relevant to garlic polysaccharide (GP)-driven host effects are short-chain fatty acids (SCFAs), microbiota-modified bile acids, and tryptophan-derived metabolites. These metabolites act as signaling molecules coupling microbial activity to host metabolic and immune responses and therefore form the mechanistic bridge between GP intake and improvements in metabolic disease phenotypes (Hou *et al*., 2023; Zhu *et al*., 2024) .

4.3.1.Short-Chain Fatty Acids (SCFAs)

SCFAs (mainly acetate, propionate, and butyrate) are produced by saccharolytic bacteria during fermentation of fermentable carbohydrates such as low-DP fructans/oligosaccharides. They have multiple host targets: they serve as energy substrates for colonocytes, modulate intestinal barrier function, signal through G-protein coupled receptors (GPR41/FFAR3 and GPR43/FFAR2), and

inhibit histone deacetylases (HDACs) to alter gene expression in immune cells. Through these routes SCFAs promote regulatory T cell (Treg) differentiation, suppress pro-inflammatory cytokine production, and improve insulin sensitivity and lipid metabolism—mechanisms that are central to metabolic disease amelioration (Facchin *et al*., 2024; D. Zhang *et al*., 2023) .

Garlic polysaccharide studies report increased fecal SCFA production following administration of low-MW/oligosaccharide fractions or processed/aged garlic saccharides. For example, water-soluble garlic polysaccharide (WSGP) supplementation in murine colitis models increased SCFAs while improving barrier proteins and reducing inflammatory signaling. Aged garlic oligosaccharides used in high-fat/high-cholesterol models similarly raised fecal acetate/propionate/butyrate and were associated with improvements in atherosclerotic and metabolic readouts. These findings support a model in which GP-driven enrichment of SCFA-producing taxa mediates downstream immune and metabolic benefits (Shao *et al*., 2024; X. Wang *et al*., 2025).

4.3.2.Bile Acids (Microbiota-modified)

Primary bile acids synthesized by the liver are metabolically transformed by gut microbes into secondary bile acids; the composition of the bile acid pool is therefore microbiota-sensitive. Bile acids are potent signaling ligands for host receptors such as FXR (farnesoid X receptor) and TGR5 (GPBAR1), which regulate lipid and glucose metabolism, intestinal barrier function, and immune cell activity. Microbiota-driven changes in bile acid metabolism can thus influence metabolic disease risk and inflammatory status (Y. Li *et al*., 2024; Zhu *et al*., 2024).

Although direct studies of garlic polysaccharides altering bile acid pools are fewer than those for SCFAs, interventions with garlic-derived oligosaccharides and related garlic preparations have been linked to microbiota shifts that plausibly reduce pro-atherogenic metabolites (e.g., trimethylamine to Trimethylamine-N-oxide TMAO) and alter bile-acid-related signaling. Aged garlic oligosaccharide supplementation in an atherosclerosis model decreased TMAO formation and was accompanied by microbial and metabolic reprogramming, suggesting that GP intake can indirectly modulate bile-acid and other microbially-derived lipid mediators relevant to cardiovascular and hepatic health (Mao *et al*., 2024).

4.3.3.Tryptophan-derived Metabolites

Microbial metabolism of tryptophan yields indoles and related compounds (e.g., indole-3-propionic acid, indole-3-aldehyde) that act on host receptors including the aryl hydrocarbon receptor (AhR). AhR activation by these microbial indoles supports mucosal barrier integrity, induces IL-22 production (important for epithelial repair and antimicrobial peptide expression), and modulates innate and adaptive immune responses—pathways implicated in metabolic and inflammatory diseases. Another fraction of tryptophan is processed via the host kynurenine pathway, which also interfaces with immune regulation; the balance of these routes is microbiota-influenced (Hezaveh *et al*., 2022; Hou *et al*., 2023).

While direct reports linking garlic polysaccharide intake to specific tryptophan-metabolite profiles are still emerging, broader polysaccharide interventions demonstrate shifts in microbial tryptophan metabolism and enhanced AhR ligand availability. Given that garlic GPs alter microbiota composition—promoting taxa that are competent in tryptophan catabolism—the mechanistic plausibility is strong that GP consumption affects immune tone via tryptophan-derived AhR ligands. Reviews summarizing microbial-Trp–AhR crosstalk recommend targeted metabolomics in future GP studies to confirm these pathways (Dai *et al*., 2021; S. Li, 2023).

4.4. Interaction with Intestinal Immunity

Garlic polysaccharides (GPs) influence intestinal immunity both indirectly (via microbially produced metabolites) and directly (via interactions with immune cells), producing measurable effects on mucosal barrier integrity

and immune cell regulation, such as regulatory T cells (Tregs) and cytokine profiles.

4.4.1.Effects on mucosal barrier integrity

Multiple in vivo studies report that water-soluble and low-molecular-weight garlic polysaccharide fractions protect or restore mucosal barrier structure in models of intestinal injury. In DSS-induced ulcerative colitis (UC) mice, supplementation with a water-soluble garlic polysaccharide (WSGP) reduced histological damage, preserved goblet cell mucin, and increased expression of tight-junction proteins (ZO-1, occludin), thereby decreasing intestinal permeability and disease activity index. These protective effects coincided with suppression of NF-κB/STAT3 inflammatory signalling and shifts in microbiota/metabolite profiles, indicating a coordinated barrier–microbiota–immune effect (Shao *et al*., 2020, 2024a).

Similar barrier-protective outcomes have been reported in other GP interventions: studies noted increased mucin staining, reduced epithelial erosion, and recovery of villus/crypt architecture after GP or garlic oligosaccharide feeding in inflammatory and metabolic models, again often parallel to rises in SCFAs and increases in SCFA-producing taxa (e.g., Lachnospiraceae, Akkermansia). These observations support the view that GP-driven microbiota changes and SCFA increases mediate much of the mucosal protection, though direct interactions between polysaccharides and epithelial or immune cells may also contribute (T. Li *et al*., 2024; Shao *et al*., 2020). Mechanistically, SCFAs produced from GP fermentation (butyrate, propionate) provide colonocyte energy, promote tight-junction assembly, and stimulate mucus production—processes that reduce translocation of microbial components and downstream systemic inflammation. Reviews summarizing SCFA biology emphasize these epithelial actions as fundamental routes by which prebiotic fibres improve barrier integrity in inflammatory and metabolic disease contexts (Ney *et al*., 2023; Venegas *et al*., 2019).

4.4.2.Regulation of Tregs and Cytokines

GP interventions modulate immune cell phenotypes and cytokine responses in both local (intestinal) and systemic compartments. In animal models, GP or garlic oligosaccharide supplementation decreased pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) and increased anti-inflammatory markers such as IL-10 and TGF-β in colon tissue and serum, aligning with improved histology and clinical scores in colitis and metabolic disease models. These cytokine shifts are consistently reported alongside microbiota/metabolite changes, highlighting the role of a microbe-metabolite-immune axis (M. Lu *et al*., 2023).

Regulatory T cells ($FoxP3^+$ Tregs) are a key cellular target of microbiota-derived metabolites. SCFAs enhance Treg differentiation and function via GPR43/GPR109A signaling and HDAC inhibition, increasing FoxP3 expression and IL-10 production—mechanisms shown in multiple preclinical studies and reviews. Although direct quantification of Treg expansion after GP administration is limited, studies that measure downstream markers (increased IL-10, reduced Th17 markers) suggest Treg-mediated immunoregulation contributes to GP benefits. Targeted studies measuring Treg frequency and function following GP treatment remain a priority (Hu *et al*., 2022; Kim, 2023). There is also evidence that certain GP fractions can directly stimulate innate immune cells: fructan-type garlic polysaccharides enhanced macrophage activation (↑NO, TNF-α, IL-6) in RAW264.7 cells and restored immune parameters in immunosuppressed mice, indicating context-dependent immunostimulatory potential that may help restore immune competence while concurrent metabolite-driven signals temper excessive inflammation in disease settings (Sun *et al*., 2025; Wu *et al*., 2024).

5.The Flora Metabolites – Immunity Axis in Metabolic Disease

Dietary garlic polysaccharides (GPs) alter gut community structure and fermentation outputs, producing metabolites that act as signalling intermediates between the microbiota

and the host immune system. These metabolite-mediated immune effects are central to how GPs can influence metabolic diseases such as obesity, type 2 diabetes (T2D), nonalcoholic fatty liver disease (NAFLD)/MAFLD, and atherosclerosis.

5.1.SCFAs and Immunomodulation in Obesity and Diabetes

Short-chain fatty acids (SCFAs)—mainly acetate, propionate and butyrate—are produced by microbial fermentation of fermentable carbohydrates such as low-DP oligosaccharides and fructans. SCFAs influence host metabolism and immunity through multiple mechanisms: they serve as energy substrates for colonocytes, engage G-protein coupled receptors (GPR41/FFAR3, GPR43/FFAR2, GPR109A), and inhibit histone deacetylases (HDACs), thereby shaping gene expression in immune cells and promoting regulatory T cell (Treg) differentiation and anti-inflammatory cytokine production. These pathways reduce intestinal permeability, systemic inflammation, and improve insulin sensitivity—mechanistic routes relevant to obesity and T2D prevention/amelioration (Kim, 2023; Van *et al*., 2024; D. Zhang *et al*., 2023).

Evidence linking SCFAs to metabolic improvements includes preclinical and clinical observations: increased colonic butyrate and propionate are associated with improved glucose homeostasis, reduced adipose inflammation, and enhanced Treg numbers in metabolic tissues (rodent models and translational human data). Multiple reviews synthesize how SCFA-driven immune regulation (Treg induction, suppressed pro-inflammatory cytokines) contributes to improved insulin sensitivity and reduced adipose/tissue inflammation in obesity and T2D contexts (Anachad *et al*., 2023; Cui *et al*., 2023). GP fractions, especially low-MW/processed oligosaccharides, increase fecal SCFAs and enrich SCFA-producing taxa in animal models, offering a plausible link from GP ingestion to SCFA-mediated immunometabolic benefits (Van *et al*., 2024).

5.2. Bile Acids and Liver

Bile acids (BAs), synthesized in the liver and modified by gut microbes into secondary BAs, function as signaling molecules that regulate lipid and glucose metabolism, energy expenditure, and immune responses via receptors such as FXR (farnesoid X receptor) and TGR5 (GPBAR1). FXR signaling in the intestine and liver controls bile acid synthesis (via CYP7A1), lipogenesis, and systemic metabolic pathways; TGR5 activation on immune and metabolic cells modulates energy balance and inflammation. Dysregulated bile acid composition and signaling are implicated in NAFLD/MAFLD progression and cardiometabolic disorders (Chiang & Ferrell, 2020; Fleishman & Kumar, 2024).

Microbiota shifts induced by polysaccharide interventions can reshape the bile-acid pool and FXR/TGR5 signaling: for example, microbial communities that favor deconjugation/7α-dehydroxylation change the ratio of primary to secondary BAs and thereby alter receptor activation patterns linked to hepatic lipid handling and systemic inflammation. In models of metabolic disease, interventions that favor beneficial bile-acid signaling (appropriate FXR/TGR5 balance) reduce hepatic steatosis, lower inflammatory markers, and improve lipid profiles (Chiang & Ferrell, 2020; Zhu *et al*., 2024). Specific to garlic preparations, aged garlic oligosaccharides and other GP fractions have been associated in animal studies with reduced TMA/TMAO (a microbial-derived pro-atherogenic metabolite) and microbiota changes that are consistent with favorable bile-acid modulation, suggesting a mechanistic path from GP to microbiota, bile-acid signalling and improved hepatic/cardiovascular outcomes. However, direct, targeted bile-acid profiling after isolated GP supplementation is still limited and remains an important research need (Zerem *et al*., 2025; Zhu *et al*., 2024).

5.3. Tryptophan Metabolites and Systemic Inflammation

Tryptophan (Trp) metabolism represents a critical microbiota–host interface. Microbial catabolism of Trp produces indole derivatives

(indole-3-propionic acid, indole-3-aldehyde, indoleacetic acid, etc.) that act as ligands for the aryl hydrocarbon receptor (AhR), promoting mucosal barrier integrity, IL-22 production, and antimicrobial peptide expression—actions that reduce epithelial inflammation and maintain immune homeostasis. The host kynurenine pathway, also influenced by microbiota and inflammation, generates metabolites that can be immunomodulatory (or immunosuppressive) and are linked to metabolic and neuroinflammatory outcomes (Miyamoto *et al*., 2024; G. Wang *et al*., 2024).

Microbial production of AhR ligands from Trp helps restrain excessive inflammation and supports epithelial repair—mechanisms relevant in obesity and metabolic endotoxemia where barrier dysfunction drives systemic inflammation. Emerging studies show that dietary fibers/polysaccharides that reshape the microbiota can increase beneficial indoles and thus indirectly promote AhR-mediated anti-inflammatory effects. Direct evidence connecting garlic polysaccharide consumption to specific Trp-metabolite shifts is still emerging, but the pathway is mechanistically plausible and supported by analogous polysaccharide interventions (Miao *et al*., 2025; G. Wang *et al*., 2024).

5.4. Integration Model

Ingestion of GP leads to selective fermentation by saccharolytic microbes (particularly when GP fractions are low-DP/soluble), leading to increased production of SCFAs and shifts in microbial taxa (Van *et al*., 2024). SCFAs promote Treg differentiation and anti-inflammatory cytokine production (e.g., IL-10), indole derivatives activate AhR supporting barrier repair and mucosal IL-22 production, and favorable shifts in microbiota reduce pro-atherogenic metabolites and alter bile-acid pools to engage FXR/TGR5 signaling that improves lipid/glucose handling. These combined immune and metabolic receptor pathways reduce tissue inflammation and metabolic dysfunction (Chiang & Ferrell, 2020; Kim, 2023).

Reduced adipose and hepatic inflammation, improved insulin sensitivity, lower atherogenic metabolite burden (TMAO), and enhanced gut barrier integrity converge to ameliorate obesity, T2D, NAFLD/MAFLD, and associated cardiovascular risk. Animal studies with GP/aged garlic oligosaccharides show concordant microbiota/metabolite/phenotype improvements consistent with this model; translational human data remain limited (Anachad *et al*., 2023; Zerem *et al*., 2025).

6.Comparison with Other Dietary Polysaccharides

Dietary polysaccharides commonly studied for prebiotic and immunomodulatory effects include inulin, fructooligosaccharides (FOS), and β-glucans. Garlic polysaccharides (GPs) share important functional similarities with these established fibers but also display unique features—particularly a dual influence on gut microbiota composition and host immunity—that merit attention.

6.1.Similarities in Prebiotic Role

Like inulin and FOS, many garlic polysaccharide fractions (especially low-DP/oligosaccharide fractions and processed/aged saccharides) are resistant to upper-GI digestion and are fermented in the colon, producing short-chain fatty acids (SCFAs) and stimulating saccharolytic taxa such as *Bifidobacterium* and *Lactobacillus*. In vitro and in vivo studies indicate that hydrolysed garlic saccharides and low-Mw GP fractions exert bifidogenic and SCFA-generating effects broadly comparable to other fructan-type prebiotics, although potency varies with DP and extraction method. Reviews of inulin/FOS consistently report strong bifidogenic activity, and garlic saccharides often fall within this functional class due to their inulin-type fructan backbone (X. Lu *et al*., 2021; Teferra, 2021). Clinical and animal literature for established prebiotics (inulin, FOS) documents benefits on gut ecology, bowel function, and metabolic markers; garlic polysaccharide studies reproduce several of these endpoints in animal and in vitro models, supporting the classification

of GPs as functional prebiotic polysaccharides when appropriately fractionated (Dou *et al*., 2022; Zhao *et al*., 2022b).

6.2. Differences and Unique Features of Garlic Polysaccharides

While inulin and many commercial FOS are relatively well-characterized linear fructans (β-(2→1) fructofuranosyl linkages) with predictable DP ranges, garlic polysaccharide extracts display greater heterogeneity. Depending on source material and processing, garlic yields both inulin-type fructans (bulb) and pectin-rich fractions (peel, leaf, pomace) containing galacturonic acid, RG-I/HG domains, and neutral sugar side chains. This structural diversity confers varied solubility, fermentability, and physicochemical properties not seen in single-source prebiotics like purified inulin (Xie *et al*., 2024; Zhao *et al*., 2022b).

6.2.1.Dual Microbiota and Immunity Impact

β-glucans are celebrated for their direct immunomodulatory activity (via Dectin-1 and other pattern recognition receptors) and also have prebiotic effects depending on source and solubility; inulin/FOS are primarily recognized for microbiota/SCFA-mediated immune benefits. Garlic polysaccharides occupy an intermediate/dual niche: many GP fractions behave as prebiotics (promoting SCFA production and beneficial taxa), and specific GP fractions show direct immunomodulatory actions (macrophage activation, cytokine modulation) in vitro and in vivo. This dual action—microbiota-mediated metabolite signaling plus direct innate immune engagement—distinguishes garlic polysaccharides from purely fermentable fibers and aligns them partially with immunoactive polysaccharides such as β-glucans (Singh & Bhardwaj, 2023; Wu *et al*., 2024).

6.2.2.Processed/Aged Garlic Products with Unique Metabolic Effects

Aged or processed garlic oligosaccharides have been reported to reduce pro-atherogenic metabolites (e.g., TMA/TMAO) and remodel microbiota in ways that directly impact cardiovascular and hepatic outcomes—effects that are not always observed with generic inulin or FOS supplementation. These specific metabolic endpoints (TMAO reduction, bile-acid-related signalling shifts) have been demonstrated in recent animal models using garlic oligosaccharides and provide mechanistic rationale for garlic's application in cardiometabolic contexts (T. Li *et al*., 2024; X. Lu *et al*., 2021).

7.Challenges and Future Perspectives

7.1.Current Limitations

Most studies on garlic polysaccharides (GPs) remain preclinical: in vitro fermentations and rodent models dominate the literature, with relatively few randomized controlled trials (RCTs) using well-characterized GP fractions in humans. Several recent reviews highlight this translational gap and call for human intervention studies that integrate microbiome and immune endpoints to validate preclinical findings (El-Saadony *et al*., 2024; Jiang *et al*., 2022). There is substantial methodological heterogeneity in how GPs are extracted, fractionated, and characterized—ranging from traditional hot-water + ethanol precipitation to enzymatic, ultrasonic/microwave-assisted, and membrane/column fractionation approaches. This diversity leads to wide differences in degree of polymerization (DP), molecular weight, branching, and composition across studies, making direct comparisons difficult and complicating reproducibility and meta-analysis. Several recent reviews and methodological papers emphasize the need for standardized extraction and reporting guidelines (Irianto *et al*., 2025; Jiménez-Amezcua *et al*., 2025). Dose-response relationships for specific GP fractions are poorly defined: animal studies use a broad range of doses, and human data—when present—often use whole garlic preparations rather than isolated, characterized polysaccharide fractions. Bioavailability of polysaccharide-derived metabolites (e.g., systemically measured SCFAs, indoles, bile-acid species) depends on fermentation kinetics and intestinal transit, but targeted pharmacokinetic data for GP fractions are scarce. Safety profiles for isolated GP fractions appear favorable in preclinical work, yet systematic toxicology and allergenicity assessments, as

well as well-controlled human safety/dose-finding trials, are lacking. Reviews of garlic bioactives also note unresolved issues around standardizing "active" dose equivalents across preparations (Lawson & Hunsaker, 2018; Shao *et al*., 2024a; Sunanta *et al*., 2023).

7.2. Future Directions

To move from associative to causal inference, future GP research must combine shotgun metagenomics (taxonomic + functional capacity) with targeted metabolomics (SCFAs, bile acids, tryptophan metabolites, TMA/TMAO) and glycomics (detailed GP structure—DP, linkage, branching). Multi-omics integration will enable mapping of which GP structures drive specific microbial metabolic pathways and immune outcomes. Recent methodological reviews highlight the power of combined metagenomics–metabolomics platforms for nutritional intervention studies and recommend standardized pipelines for reproducibility (Aya *et al*., 2025; Yang *et al*., 2025). Interindividual variability in baseline microbiota composition strongly influences response to dietary fibers and prebiotics. Personalized or stratified approaches—using baseline microbiome signatures to predict who will respond to a given GP fraction—could substantially improve efficacy of interventions for metabolic disease. Reviews and pilot studies in personalized diets and microbiome stratification provide frameworks that should be applied to GP trials (e.g., enterotype-informed supplementation, responder/non-responder analyses) (Hernández-Calderón *et al*., 2022; Song & Shin, 2022). For translational impact, GPs must be developed into standardized ingredients (with defined DP/Mw profiles) and formulated into food matrices or nutraceuticals with demonstrated stability, palatability, and shelf life. Process innovations (green extraction, membrane fractionation, microencapsulation) can improve yield and bioavailability while keeping structural integrity. Regulatory, scalability, and cost considerations will also be central to bringing GP-based products to market. Recent reviews on extraction innovation and food-component characterization outline practical steps for industrial translation (Irianto *et al*., 2025). Well-designed human RCTs should: (1) use chemically characterized GP fractions (with DP, Mw, and linkage data), (2) include multi-omics endpoints (microbiome, metabolome, immunophenotyping), (3) incorporate dose-finding and safety arms, and (4) stratify participants by baseline microbiome or metabolic phenotype to identify responders. Such studies will be essential to demonstrate causality and to support health claims or therapeutic uses in metabolic disease. Method papers and reviews stress the importance of these integrated designs for dietary-microbiome interventions (Chinta *et al*., 2025; Yang *et al*., 2025). Finally, creating community standards for reporting extraction/purification methods, structural characterization, and bioactivity assays (analogous to CONSORT for clinical trials) will improve comparability. Collaborative consortia that share standardized reference GP materials and harmonized protocols will accelerate progress and reduce duplication. Reviews and editorials advocating for standardization across microbiome–nutrition research provide blueprints for such efforts (Falsafi *et al*., 2025; Yang *et al*., 2025).

8.Conclusion

Garlic polysaccharides (GPs) represent an emerging class of non-sulfur bioactives with dual roles as prebiotics and immunonutrients. Their structural heterogeneity spanning fructan- and pectin-type fractions shapes fermentability, microbial selectivity, and downstream production of key metabolites such as short-chain fatty acids, bile acids, and tryptophan derivatives. Through these microbiota–immune interactions, GPs exert antioxidant, anti-inflammatory, immunomodulatory, and metabolic benefits in preclinical models of obesity, diabetes, fatty liver disease, and atherosclerosis. Compared with established dietary polysaccharides, GPs show distinctive potential by coupling microbiota-driven metabolite signalling with direct immunological effects. However, translation remains limited by methodological variability, insufficient structural standardization, and a lack of well-

controlled human clinical trials. Future work should focus on multi-omics integration, dose–response characterization, and personalized nutrition strategies to confirm efficacy and enable the development of GP-based functional foods or therapeutics.

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No new data were created or analyzed in this study. Data sharing does not apply to this article.

Declarations

Ethics approval and consent to participate

The authors declare that they have no human and/ or animal studies in this manuscript.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.