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PECTIN OLIGOSACCHARIDES IS A PROMISING PREBIOTIC FUNCTIONAL FOOD

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ABSTRACT

Pectin oligosaccharides (POS) are oligosaccharides derived from the degradation of natural pectin and have garnered considerable attention due to their notable bioactivities. This review presents a comprehensive overview of recent developments concerning the structure, preparation methods, and biological activities of POS. It particularly highlights the roles of POS in anti-inflammatory responses, immune modulation, lipid metabolism regulation, gut microbiota composition alterations, and anticancer potential, while also emphasizing their promising applications in the field of food science. By elucidating the prebiotic activities and underlying mechanisms of POS, this review aims to provide valuable insights for future research and to facilitate the early application of POS as functional food ingredients.

Keywords: Pectin oligosaccharides; prebiotics; intestinal flora; functional food

INTRODUCTION

Functional foods are defined as dietary components that, when consumed at physiologically relevant levels as part of a balanced diet, confer targeted health benefits beyond basic nutrition.**(Granato *et al.*, 2020)** Common examples of functional foods include prebiotics, which are thought to enhance the survival, growth, metabolism, and health-promoting activities of probiotics within the digestive system. The most widely consumed prebiotics include lactulose, galacto-oligosaccharides, fructooligosaccharides (FOS), xylo-oligosaccharides, and inulin, along with their hydrolysates, which are non-active constituents in the diet **(Granato *et al.*, 2020)**. The primary site of action for prebiotics is the colon, where they are believed to regulate gut microbiota through dietary means **(Sanders *et al.*, 2019)**. Indeed, numerous studies have demonstrated that various prebiotics may have potential applications in the management and treatment of different diseases **(Kennedy *et al.*, 2024; Peng *et al.*, 2020)**.

Pectin is a complex macromolecular polysaccharide that is widely found in the cell walls of certain fruits and vegetables, including oranges, lemons, apples, and pumpkins **(Liang *et al.*, 2022)**. It is extensively utilized in the food industry **(Thakur *et al.*, 1997)**, and numerous investigations have been conducted in the fields of nutritional healthcare **(Naqash *et al.*, 2017)** and biomedicine **(Munarin *et al.*, 2012; Noreen *et al.*, 2017)**. Pectin oligosaccharide (POS) is an oligosaccharide produced through the decomposition of natural pectin. The potential prebiotic properties of POS have garnered ongoing interest. Earlier studies have indicated that hydrolyzed or enzymatically digested pectin exhibits prebiotic effects **(Foti *et al.*, 2022; Olano-Martin *et al.*, 2002)**. Toxicological studies further suggest that POS is a safe and indigestible carbohydrate **(Garthoff *et al.*, 2010)**. Subsequent research has confirmed that POS can specifically increase the populations of bifidobacteria, lactobacilli, and enterococci in *in vitro* fecal fermentation experiments, highlighting its role in regulating gut microbiota **(Gómez *et al.*, 2014; Manderson *et al.*, 2005)**. Collectively, these findings suggest that POS can be effectively utilized as a prebiotic. Additionally, POS has shown promise as a prebiotic product in agriculture and animal nutrition **(Babbar *et al.*, 2016; Wilkowska *et al.*, 2020)**. However, the biological properties and mechanisms of POS remain inadequately studied, which limits its advancement into practical applications in food science. This review summarizes current research on the potential prebiotic activity of POS in the biomedical field and discusses its bioactivity and possible mechanisms.

THE PREPARATION AND STRUCTURAL CHARACTERISTICS OF POS

Pectin is a family of galacturonic acid-rich polysaccharides, which includes homogalacturonan, rhamnogalacturonan I, and the substituted galacturonans rhamnogalacturonan II and xylogalacturonan. **Figure 1** illustrates the structural diversity of pectin. In addition to the three main polysaccharides, arabinogalactan, arabinogalacturan, and xylogalacturonoglycan are also present in natural pectin **(Mohnen, 2008)**. Furthermore, pectin exhibits a significant degree of structural complexity at the secondary and tertiary levels **(Zdunek *et al.*, 2021)**. As a hydrolysate of pectin, POS can be regarded as oligosaccharides composed of galacturonic acid. The typical composition of POS consists of 2 to 10 units of pectic acid, which are linked by α-1,4-glucoside bonds; however, additional structural elements may also be present. These branched chains typically comprise other monosaccharides, such as arabinose, xylose, and galactose, which can enhance their biological activity and functional properties. Additionally, the ingredients of POS preparations are predominantly derived from natural plants or food industry residues, which consequently increase the monosaccharide diversity of POS products **(Babbar *et al.*, 2016)**. Pectin oligosaccharides have relatively low molecular weights, typically ranging from several thousand to tens of thousands of Daltons (Da) **(Babbar *et al.*, 2016)**, facilitating their accessibility within the digestive system while preserving their prebiotic properties **(Tingirikari, 2018)**.

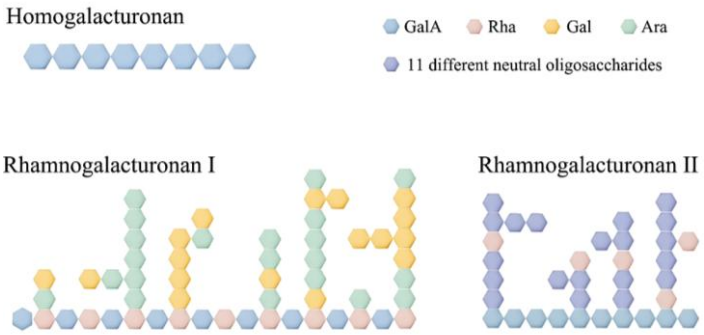

Figure 1 Schematic diagram of the three basic pectin structures.

The homogalacturonan (HGA) domain is a polymer of D-galacturonic acid linked by an α-1,4-glucoside bond. The RG-I domain is diverse and branched and is often called the hairy region. The main chain is made of up to 100 or more repeating units of disaccharide. The disaccharide unit is made of L-rhamnose and D-galacturonic acid, connected by α-1,4 and α-1,2 bonds. The RG-II domain is compact and consists of an HGA skeleton with nine galacturonic acid units, to which four distinct chains are attached. (GalA: galacturonic acid; Rha: rhamnose; Ara: arabinose; Gal: galactose) **(Christiaens *et al.*, 2016; Mohnen, 2008; Zdunek *et al.*, 2021)**

With the gradual enrichment of biological studies on POS, an increasing number of preparation schemes for POS have been proposed. **Table 1** summarizes the principal methods for POS preparation.

The predominant methodology for POS production entails enzymatic hydrolysis of pectin substrates using pectinase complexes, often augmented with auxiliary enzymes (e.g., cellulases, xylanases) to enhance depolymerization efficiency. The most prevalent protocol involves the preparation of POS through the cleavage of pectin using conventional pectinase **(Vásquez *et al.*, 2024; Zhu *et al.*, 2019)**, which also includes the use of complex polysaccharide enzymes **(Prandi *et al.*, 2018)**. The primary component obtained from POS is galacturonic acid, accompanied by minor amounts of arabinose and rhamnose. However, the products derived from POS preparation can vary due to the diverse raw materials used. Other researchers have utilized genetic engineering to culture bacteria that produce lyases, which act on substrates to generate POS **(Zheng *et al.*, 2021; Zheng *et al.*, 2022)**. In addition to enzymatic hydrolysis, POS can also be synthesized through chemical methods that employ inorganic reagents, such as acids or bases **(Cano *et al.*, 2021; Zhang *et al.*, 2018)**. An earlier study indicated that POS can be produced by cleaving pectin through UV irradiation, a purely physical method **(Burana-osot *et al.*, 2010)**. In light of these studies, researchers have combined enzymatic reactions with physical or chemical methods, leading to the finding that the products of enzymatic hydrolysis can be effectively regulated by pretreatment prior to enzymatic hydrolysis **(Manthei *et al.*, 2024; Wilkowska *et al.*, 2019)**. Agnieszka Wilkowska et al. reported that following mild acid hydrolysis, pectinase was utilized to decompose pectin, resulting in a relatively high concentration of POS. In contrast, the application of pure enzymatic processing of the substrate using a mixture of cellulase and pectinase led to a 1.8-fold reduction in total POS. However, the concentration of higher-order oligosaccharides (degree of polymerization 7-10) in the POS products was found to be 1.7 times greater **(Wilkowska *et al.*, 2019)**. In another study, Min Yu and colleagues combined physical methods with enzymatic hydrolysis, obtaining a homogeneous POS with a molecular weight of 2.15 kDa via enzymatic hydrolysis following ultrasonic pretreatment of finger citrate residue **(M. Yu *et al.*, 2021)**. A further set of studies compared high-pressure homogenization (HPH), enzymatic hydrolysis (EH), and their combination (HPHE). Researchers reported that, compared to orange peels, the pectin of apple peels interacts more extensively with the surrounding (semi-)cellulose matrix, which is a disadvantage in the context of enzymatic hydrolysis. Therefore, employing a physical pretreatment method can significantly enhance the efficiency of enzymatic hydrolysis for the production of POS **(Manthei *et al.*, 2024)**.

Notably, POS products exhibit significant diversity, attributed to the variety of raw materials and preparation schemes employed. Unlike certain other functional foods that possess specific components and a fixed structure, identifying the exact components of a POS product proves challenging. This observation aligns with the diversity of major components and molecular sizes noted in the research results presented in **Table 1**. Nevertheless, this complexity and diversity also present opportunities for enhanced biological activity and functional properties of POS.

Table 1 Representative POS preparation methods.

Ingredients	Methods	Enzyme	Reagents / Auxiliaries	Main monosaccharide or oligosaccharide components	Product Molecular weight (Da) / Degree of polymerization (DP)	Ref.
Citrus peel pectin	Alkaline hydrolysis (pH=10)		H_2O_2, trifluoroacetic acid (TFA)	l-rhamnose, d-galacturonic acid, d-glucose and d-galactose	3628 Da, 2673 Da, 3543 Da, 2661 Da	**(Zhang *et al.*, 2018)**
Commercial pectin, orange peel and apple pomace	Continuous acid treatment		HCl, TFA	Galacturonic acid (GalA), arabinose (Ara), mannose (Man), galactose (Gal), rhamnose (Rha)	Solid 3 (Medium POS): DP 3~14 Solid 2 (Large POS): DP 14~55 。 Solid 4 (Small POS): DP 2~8	**(Cano *et al.*, 2021)**
Hawthorn	Enzymolysis	pectinase		GalA (72.3%), Ara (17.3%), Rha (3.6%)	LM-POS < 700 Da 700 Da < MM-POS < 3000 Da HM-POS > 3000 Da,	**(Zhu *et al.*, 2019)**
Sugar beet pulp	Enzymolysis	Viscozyme		Ara, Polygalacturonans, Rhamnogalacturonans	400 Da~5000Da	**(Prandi *et al.*, 2018)**
Pisco grape pomace	Enzymolysis	Pectinase (Pectinex Ultra SP-L)		POS 1: GalA (with high glucose content) POS 2: GalA (with minor other monosaccharide) PF: GalA (High galactose-fructose content); Ara, Rha	POS 1: <3 kDa POS 2: 3 kDa~10 kDa PF: >10 kDa, with partially esterified GalA chain	**(Vásquez *et al.*, 2024)**
Sodium polygalacturonate	Enzymolysis	ErPL2 (Pectate lyase, from Echinicola rosea JL3085)			oligogalacturonic acid (1 to 5 polymers)	**(Zheng *et al.*, 2022)**
Pectin from citrus peel, and pectin from apple	Enzymolysis	ErPL2			DP 1-6, DP 2-3 predominates, indicating that these products are 4,5-unsaturated oligogalacturonic acid	**(Zheng *et al.*, 2021)**
Apple pomace	Enzymolysis	Pectinase, cellulase	HCl (for acidolysis)		Main DP 2~DP 9	**(Wilkowska *et al.*, 2019)**
	Mild acid hydrolysis + enzymatic hydrolysis (two-step process)	polygalacturonases, pectin lyases, and cellulases (Cellulosoft+Viscozyme; Rohament CL+Rohapect Ma Plus T)			Higher outputs, with higher-order oligosaccharides (DP 7-9) Reduced	
Finger citron pomace	Enzymolysis + ultrasound assistance	Pectinase	Ultrasonic	Man, GalA, Gal, Ara	2.15 kDa homogenous oligosaccharides	**(M. Yu *et al.*, 2021)**

	High-pressure homogenizatio n (HPH),		Ultrasonic homogenization and high-pressure homogenization	DP 1-2: hexose (e.g., glucose, sucrose, cellobiose) DP 2-4: arabinoxylans, substituted with galactose and some ferulic acid DP 5: Linear pentose (arabinose or xylose) DP 1-3 GalA, wirh pentose or hexose	Orange peels produce more POS	
Apple bagasse and orange peel	Enzymatic hydrolysis (EH)			DP 2: deoxyhexosaccharide (rhamnose) DP 3-5: Pentose with GalA units	DP 2-4 from orange peel was higher, with limited hydrolysis from apple peel.	**(Manthei *et al.*, 2024)**
	HPH+EH	Cellulase (Celluclast 1.5 L), pectinase (Pectinase 62 L)	Ultrasonic homogenization and high-pressure homogenization	GalA of DP 2-5 is more diverse in apples than oranges. All identifiable oligosaccharides are linear GalA POS with a high degree of methylation and contain acetylation in the apple residue	HPH treatment increased apple bagasse outputs, but not as much as orange peel.	
Macromolecular pectin	Photochemical reaction		Ultraviolet light, titanium dioxide (TiO2)	Galacturonic Acid, DP 1-3	Average molecular weight decreased from 400 kDa to 200 kDa, with 20 kDa (2%), 11 kDa (8%) and 5 kDa (6%)	**(Burana-osot *et al.*, 2010)**

ANTI-INFLAMMATORY IMMUNE ACTIVITY OF POS

Similar to the considerable attention garnered by the anti-inflammatory and antimicrobial properties of pectin **(Cao *et al.*, 2024; Ciriminna *et al.*, 2020)**, POS also exhibit strong anti-inflammatory and immune-modulatory activities. This is regarded as a significant attribute of POS, particularly in its role as a prebiotic compound. **Figure 2** illustrates the anti-inflammatory activity of POS.

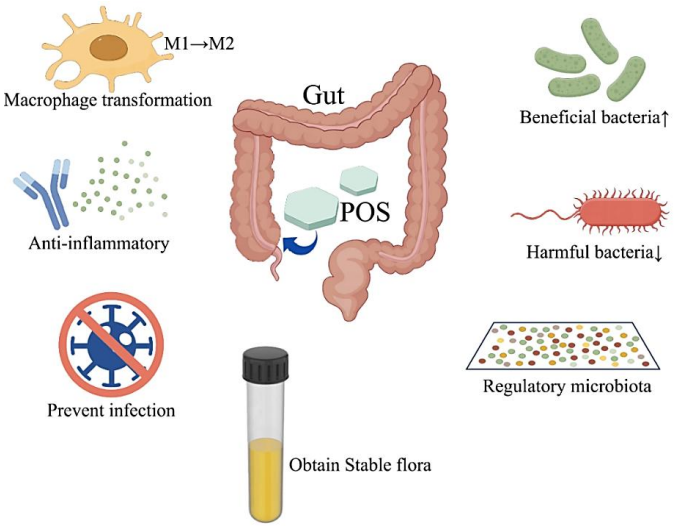

Figure 2 A summary of the anti-inflammatory immune activity of POS.

As a prebiotic, POS can facilitate anti-inflammatory effects by augmenting the growth of beneficial flora. An *in vitro* study demonstrated the capacity of *Eubacterium eligens* DSM3376 and certain strains of *Faecalibacterium prausnitzii* to utilize pectin and its degradation products as a source of prebiotics. Additionally, the researchers discovered that *E. eligens* is capable of inducing tumor necrosis factor α (TNF-α) and substantial quantities of interleukin (IL)-10, both of which exert a robust anti-inflammatory effect **(Chung *et al.*, 2017)**. Prior research has established that *F. prausnitzii* possesses anti-inflammatory characteristics **(Rossi *et al.*, 2016)**. These findings indicate that pectin and its derivative, POS, have the potential to restore equilibrium of the gut microbiome, thereby exerting anti-inflammatory effects.

In addition to regulating beneficial bacteria, POS have been shown to inhibit the destruction of harmful bacteria. Two studies have demonstrated that POS exerts an inhibitory effect on Shigella toxins. Prior research indicates that POS possesses superior preventive and mitigating effects on Shigella toxins compared to pectin **(Olano-Martin *et al.*, 2003)**. In light of these findings, Rong Di et al. utilized HT29 cells to substantiate the protective impact of POS against Shigella toxins. Researchers have reported that POS exhibits a degree of anti-adhesion activity against Shiga toxin-producing *Shigelicin E. coli* O157:H7 in HT29 cells and reduces Stx2 rRNA depurination in these cells. Furthermore, the authors indicated that POS effectively inhibits the binding of StxB subunits to Gb3 receptors on cells and may also impair the function of Stx2, thereby reducing its cytotoxicity **(Di *et al.*, 2017)**. In addition to its effects on Shigella, POS can inhibit the invasion of *Campylobacter jejuni*, with this inhibitory effect being dependent on the methodology employed for measurement **(Ganan *et al.*, 2010)**. These results demonstrate the inhibitory effect of POS on harmful bacteria. Importantly, this inhibition is highly specific; it does not rely on regulating competition between microbial populations but is instead determined by the biochemical properties of the drug itself. In other words, POS can be considered a drug rather than a typical prebiotic in this context. Further reports are needed to ascertain whether such phenomena are prevalent among gut microbes, similar to antibiotics, or if they are rare occurrences.

The impact of POS on flora extends beyond a single type and is also observable at the microbial species level. A study investigating the effects of POS on intestinal flora revealed that POS can increase the relative abundance of Bacteroidetes in a dose-dependent manner while inhibiting the proliferation of *Firmicutes*. Additionally, a dose-dependent increase in the relative abundances of *Bifidobacterium* and *Lactobacillus* was observed, whereas a corresponding decrease was noted for *E. coli* and *Enterococcus*. Furthermore, the POS processing groups enriched the highly butyrogenic Clostridium cluster XIVa **(Zhang *et al.*, 2019)**. Another study indicated that POS can exert a protective effect against colitis by altering the relative abundance of gut microbiota. Specifically, POS treatment has been shown to reduce the relative abundance of *Firmicutes* while markedly increasing the percentage of *Bacteroidota*. This alteration results in a decrease in the F/B ratio and a reduction in the percentage of *Campylobacterota*. Notably, the numbers of Bacilli, *Campylobacterales*, and *Lactobacillaceae* all increased significantly **(H. Wang *et al.*, 2022)**. These findings suggest that the anti-inflammatory effects of POS manifest through the restructuring of gut microbiota, exemplifying the prebiotic attributes of POS.

The potential exists for the creation of stable and transferable flora through the use of POS. An experiment utilizing a fecal transplantation technique corroborated the hypothesis that POS facilitates the establishment of a transplantable, high-IgA phenotypic microbiome. Researchers initially confirmed the specificity of oral POS for IgA elevation through a fecal enzyme-linked immunosorbent assay (ELISA). Subsequently, they conducted fecal microbiota transplantation (FMT) to achieve the horizontal transfer of intestinal flora with elevated IgA levels among coreceptors. This phenomenon of complete transfer also unexpectedly manifested between cobred male and female mice and their offspring, indicating that the high-IgA microflora generated by POS could be transferred vertically. In conclusion, the ileal etho-resistant microbial community, represented by *Lachnospiraceae bacterium* A2, may drive the high IgA phenotype, which is associated with the long-term induction of POS. **(Zhang *et al.*, 2023)** These findings suggest that long-term dietary modifications, such as the incorporation of POS, may facilitate the enrichment of pivotal microbial species within the microbiome. This, in turn, can be integrated with mucosal immunity to achieve a sustained and robust immune response. The findings of this study serve as a valuable reference for the application of POS, confirming that reasonably regulated POS interventions have

the potential to create a stable and beneficial microbiome. The ability of POS to create stable microbiota may offer new perspectives for clinical applications and provide guidance for the clinical implementation of FMT combined with prebiotics. **(Ooijevaar *et al.*, 2019; Porcari *et al.*, 2023)**

One study indicated that POS has the potential to be utilized as a prophylactic measure against virus-like infections. Researchers have investigated the preventive effects of POS in a Poly I:C-induced mouse model that mimics viral infection. Compared to the control group, the concentrations of the pro-inflammatory cytokines IL-5, IL-6, IL-13, and IL-17 were markedly lower following Poly I:C stimulation in the mice that were fed POS. Following POS intervention, there was a notable increase in sIgA levels in the feces and IgA and IgG levels in the plasma. Additionally, the levels of acetic acid and butyric acid in the gut of the POS group increased, whereas the levels of propionate decreased. Furthermore, POS facilitated the proliferation of *Lactobacillus*, *Prevotella, Rikenellaceae*, and *Lachnospiraceae* bacteria, which are positively correlated with the production of anti-inflammatory cytokines and SCFAs **(Sori *et al.*, 2022)**. It may, therefore, be surmised that POS has the capacity to stimulate specific groups of intestinal bacteria, activate mucosal immunity, and thus enhance preventive immunity against viral infections.

POS has been shown to enhance the expression of factors related to inflammatory processes. To investigate the effect of POS on dextran sulfate sodium (DSS)-induced intestinal inflammatory responses, the researchers measured the levels of pro-inflammatory and anti-inflammatory cytokines in both serum and colon tissue. The administration of POS resulted in a significant reduction in the levels of TNF-α, IL-1β, and IL-6 in both colon tissue and serum. Conversely, POS supplementation led to a notable increase in IL-10 levels in colonic tissue and serum. The mRNA expression levels of TNF-α, IL-1β, IL-6, and interferon-gamma (IFN-γ) mirrored these findings. Furthermore, POS has been demonstrated to enhance the balance of Treg/Th17 cells by increasing the proportion of Treg cells while decreasing the proportion of Th17 cells, thus maintaining immune homeostasis within the intestine **(H. Wang *et al.*, 2022)**. Additionally, another study indicated that POS derived from navel orange peels can significantly increase the secretion of nitric oxide (NO), TNF-α, and IL-6, and may also stimulate TLR4 receptors to activate macrophages **(T. Wang *et al.*, 2022)**. These findings illustrate an additional mechanism of immune efficacy associated with POS. Rather than solely acting through gut flora, POS appears to regulate cytokines and immune cells within the body to exert anti-inflammatory effects.

Additionally, POS can be considered a regulator of macrophage immune metabolism. Macrophages can be classified into two primary types: M1 and M2. Both types participate in the inflammatory response, with M1 macrophages primarily contributing to a pro-inflammatory response, while M2 macrophages play a crucial role in anti-inflammatory processes **(Yunna *et al.*, 2020)**. A study conducted by Haijuan Hu and colleagues investigated the immunomodulatory effects of POS and its microbial metabolites on human macrophages using citrus-derived POS. The results indicated that POS decreased the expression of TNF-α and IL-6 in macrophages, while increasing the expression of IL-10. Furthermore, the expression level of nuclear factor kappa-B (NF-κB) was significantly reduced. Additionally, the gene expression levels of two specific M2 markers, CD206 and CD163, were analyzed, revealing that the expression of CD206 increased by 11.63-fold in response to POS, with similar changes observed for CD163. Based on these findings, it can be inferred that POS and its metabolites are linked to the phenotypic transformation of macrophages between M1 and M2 **(Hu *et al.*, 2021)**. Macrophages play a pivotal role in the development of atherosclerotic plaques, and alterations in macrophage metabolism serve as reliable indicators of atherosclerosis **(Koelwyn *et al.*, 2018; Libby *et al.*, 2011)**. These results demonstrate that POS has the potential to regulate macrophage phenotype and immune metabolism, suggesting that POS may function as a prebiotic for the prevention and treatment of atherosclerosis.

Table 2 The intestinal anti-inflammatory effects of POS are summarized below.

Anti-inflammatory immune activity	Details	Effects	Ref.
Beneficial bacteria increase	Eubacterium eligens DSM3376↑, Faecalibacterium prausnitzii↑	Promote the growth of beneficial bacteria, anti-inflammatory	**(Chung *et al.*, 2017)**
Harmful bacteria Reduction	Shigella↓, Campylobacter jejuni↓	Inhibit the invasion of harmful bacteria	**(Olano-Martin *et al.*, 2003)** **(Di *et al.*, 2017)**
Remodeling gut microbiota	Relative abundance: Firmicutes↓, Bacteroidota↑, F/B↓ Bifidobacterium↑, Lactobacillus↑, E. coli↓, Enterococcus↓	Improves gut flora and treat colitis	**(Zhang *et al.*, 2019)** **(H. Wang *et al.*, 2022)**
Establish transferable microbiota	Oral POS may create a stable high IgA microbiome with horizontal and vertical transfer Lachnospiraceae A2 bacteria may drive the high IgA phenotype	Transferable microbiome can integrate with mucosal immunity for sustained and robust immune responses	**(Zhang *et al.*, 2023)**
Prevent virus-like infections	IL-5, IL-6, IL-13, IL-17↓ sIgA in feces↑, IgA and IgG in plasma↑ SCFAs like acetate and butyrate↑,propionate ↓	Regulating gut microbiota, enhancing mucosal immunity, and reducing inflammation may help prevent viral infections	**(Sori *et al.*, 2022)**
Regulating inflammatory response	colon tissue and serum: TNF-α, IL-1β, IL-6 ↓, IL-10 ↑ mRNA expression of TNF-α, IL-1β, IL-6, and IFN-γ ↓ Treg cells ↑, Th17 cells ↓ Secretion of NO, TNF-α, and IL-6 ↑, activating TLR4 receptors and stimulating macrophages	Regulating cytokines and immune cells to exert anti-inflammatory effects	**(H. Wang *et al.*, 2022)** **(T. Wang *et al.*, 2022)**
Regulating macrophages	TNF-α，IL-6↓，IL-10↑ NF-κB↓ CD206, CD163 ↑: Promotes M2 macrophage phenotype, suggesting M1 to M2 conversion	Regulates macrophage immune response and metabolism	**(Yunna *et al.*, 2020)** **(Hu *et al.*, 2021)**

Furthermore, studies have indicated that probiotics can effectively prevent urinary tract infections caused by the uropathogenic *Escherichia coli* CFT073 **(Sun *et al.*, 2019)**. These findings suggest that probiotics can impede the formation of bacterial quiescent states and reduce the prevalence of persistent cell populations generated by the uropathogenic strain CFT073. The presented findings exemplify the action of probiotics outside the intestine, indicating their potential applications beyond the gastrointestinal tract.

Interestingly, there are analogous studies in the field of food science. A study focused on the development of anti-inflammatory fish products demonstrated that POS exerts an anti-inflammatory effect through Maillard-type glycation, leading to a significant reduction in the secretion of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β. The researchers showed that POS could inhibit the expression of the CD14 gene in macrophages stimulated by LPS, thereby reducing inflammatory responses in the TLR4 - myeloid differentiation factor 88 (MyD88) signaling pathway. However, the application of POS does not affect the available lysine or induce browning **(Li *et al.*, 2025)**. Consequently, as a dietary prebiotic, the use of POS is beneficial for the development of anti-inflammatory functional foods.

In general, the anti-inflammatory immune system of POS is multifaceted and complex. However, the presence of various potential mechanisms and biological activities suggests that POS may serve as a promising prebiotic agent, providing a means to prevent and control inflammatory diseases through its application as a functional food.

THE IMPACT OF THE POS ON LIPID METABOLISM

Adipose tissue metabolism

It has been demonstrated that POS can influence the body's lipid metabolism. One study revealed that POS enhances lipid metabolism in white adipose tissue (WAT) in mice. Researchers have reported that POS decreases serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels, and markedly reduces serum triglyceride (TG) and free fatty acid (FFA) levels in mice fed a high-fat diet. Additionally, POS administration was observed to inhibit the increase in TNF-α and IL-6, while concurrently downregulating the expression of the macrophage marker CD68 gene in WAT. Furthermore, the impact of POS on genes associated with TG synthesis and deposition in WAT was examined. These findings revealed that POS intervention significantly reduced the expression of DGAT1 and SCD1 mRNAs. In conclusion, the authors highlighted that POS activates the ADPN-mediated AdipoR1/AMPK/ACC and AdipoR1/AMPK/PPARα signaling pathways in adipose tissue by promoting the expression of AdipoR1 and other genes, effectively regulating the synthesis of fatty acids and triglycerides, as well as oxidative catabolism in adipose tissue **(Fan *et al.*, 2022)**. Additionally, experiments have indicated that POS may enhance linoleic acid synthesis and improve lipid metabolism by upregulating 9,10-DHOME ((12Z)-9,10-dihydroxyoctadec-12-enoic acid), whereas pectin does not **(Zhang *et al.*, 2025)**. These studies demonstrate how POS affects adipose tissue and suggest that POS may serve as an effective means of preventing and improving lipid metabolism disorders.

Cholesterol metabolism

The consumption of POS has been shown to reduce cholesterol levels by modulating the composition of the intestinal microbiota. The authors initially established that a diet rich in POS leads to an increase in the levels of Bacteroidetes while concurrently decreasing the relative abundance of *Firmicutes*. Furthermore, there was a positive correlation between the relative abundances of *Lactobacillus* and *Bifidobacterium* and the concentration of POS. Additionally, POS supplementation resulted in elevated concentrations of acetic, propionic, and butyric acids in both fecal and cecal contents. The authors further observed that POS consumption inhibited the accumulation of total cholesterol (TC) in the liver, with levels of liver CYP7A1 and HMGCR, the rate-limiting enzymes of cholesterol synthesis, being significantly higher in the POS group compared to the control group. Pearson correlation analysis indicated a significant negative correlation between the observed relative abundances of *Bifidobacterium* and Bacteroides and serum TC concentration, alongside a significant positive correlation between these bacteria and liver CYP7A1 levels. Moreover, the fecal concentrations of propionate and butyrate were positively correlated with liver CYP7A1 levels and negatively correlated with liver HMGCR levels. These findings suggest that the inhibition of cholesterol synthesis following POS ingestion may be mediated by metabolites produced by gut microbiota **(Hu *et al.*, 2019)**. An additional experiment utilizing the Illumina MiSeq sequencing method was conducted to investigate the alterations in the structure of intestinal flora and metabolite composition following the addition of POS via *in vitro* intestinal flora fermentation. This experiment supports the hypothesis that the regulatory mechanism of cholesterol metabolism is influenced by cholesterol-associated intestinal flora and specific metabolites. Notably, POS promoted the proliferation of cholesterol-associated bacterial groups, including *Bacteroidetes*, *Bifidobacterium*, and *Lactobacillus*. *Furthermore*, it elevated the concentrations of four metabolites related to cholesterol metabolism: adenosine monophosphate, cyclic adenosine phosphate, guanosine, and butyrate, which were identified through non-targeted metabolomics and short-chain fatty acid (SCFA) analysis **(Hu *et al.*, 2024)**. Previous research has indicated that probiotics may effectively lower serum cholesterol levels **(Horáčková *et al.*, 2018)**. Additionally, several studies have shown that probiotic supplements can reduce total cholesterol and low-density lipoprotein (LDL) levels **(Sun & Buys, 2015)**. These findings imply that POS could serve as a promising substrate for the regulation of cholesterol metabolism. However, further clinical studies are necessary to clarify the specific regulatory pathways through which POS influences cholesterol metabolism.

Preventing and treating fatty liver disease

Hepatic steatosis is characterized as a pathological condition marked by fat accumulation in the liver, which can arise from various factors, including obesity and abnormal lipid metabolism **(Eslam *et al.*, 2020; Wen *et al.*, 2022)**. A study conducted by Shengnan Yu and her colleagues demonstrated that POS effectively improved obesity and hepatic steatosis induced by a high-fat diet (HFD). The researchers reported that HFD leads to hepatocyte swelling and steatosis in mice, accompanied by significant increases in ALT, AST, TC, and TG levels. However, after POS supplementation, hepatocyte swelling was notably reduced, and the degree of fatty liver degeneration was partially alleviated. Furthermore, POS treatment significantly reversed the elevation in ALT and markedly reduced liver TG content. These findings suggest that POS supplementation may serve as an effective strategy for mitigating HFD-induced hepatic steatosis. Additionally, following POS supplementation, the expression levels of TNF-α, IL-1β, and IL-6 in the liver decreased, while IL-10 expression increased, indicating a reduction in local liver inflammation in mice. The effects of POS on specific metabolic pathways in the liver were also evaluated. The researchers found that POS supplementation effectively reversed the low mRNA levels of downstream factors of the peroxisome proliferator-activated receptor γ (PPAR-γ) pathway, which had been suppressed by HFD **(Yu *et al.*, 2023)**. Activation of the PPAR-γ pathway has been shown to diminish inflammatory processes and ameliorate metabolic disorders associated with obesity **(Chen *et al.*, 2018)**. It can be posited that PPAR-γ may be implicated in the mechanism of POS, playing a pivotal role in its effects. This finding aligns with results from animal experiments, which demonstrated that POS supplementation reduces lipid accumulation in liver cells **(Yu *et al.*, 2023)**. Another experiment provided further insights into the potential mechanisms through which POS influences lipid metabolism. Researchers analyzed the levels of adiponectin (ADPN), AdipoR1, LKB1, and AMPKα in mouse livers. In addition to modulating the mRNA and protein levels of AdipoR2 and PPARα, POS functions as an AdipoR agonist, affecting downstream signaling in response to ADPN stimulation via the AdipoR1/AMPKα/LKB1 and AdipoR2/PPARα pathways, thereby enhancing lipid oxidation in the liver. Furthermore, the research team conducted a study on mitochondria within the livers of mice. POS administration significantly elevated the mRNA levels of ACO and CPT-1, which are crucial for fatty acid oxidation, while concurrently reducing the expression of ACC. Simultaneously, the expression of NRF1 and Tfam in mouse liver mitochondria increased, resulting in improved mitochondrial function **(Q. Yu *et al.*, 2021)**. These findings suggest that POS holds considerable potential for preventing fatty liver disease. However, fatty liver disease is a complex metabolic condition that poses significant challenges to human health, and its mechanisms are likely multifaceted. The present study provides preliminary insights into the efficacy of POS in treating fatty liver disease and suggests several potential avenues for further investigation. Additional studies are necessary to determine the long-term efficacy of POS in managing fatty liver and the effects of multidrug combinations

POTENTIAL EFFECTS OF POS ON INTESTINAL MICROMORPHOLOGY

Numerous experiments have documented the impact of POS on the morphology and functionality of intestinal microtubules. One study examining the effects of POS on the gut immune system revealed that the addition of POS had a minimal effect on gut crypt depth, while the villus height experienced a slight reduction. Additionally, the number of goblet cells increased in a dose-dependent manner. The addition of POS also results in a notable increase in the weight ratio of the colon to the intestinal contents, which is accompanied by an elevated concentration of butyric acid in the intestine. **(Zhang *et al.*, 2019)**. Another experiment investigated the anti-colitis effect of POS induced by DSS. The results demonstrated that POS significantly alleviated DSS-induced colon shortening and weight loss. H&E staining revealed that POS significantly reduced histological damage, particularly by promoting the recovery of intact mucosal epithelium and decreasing inflammatory cell infiltration and glandular damage **(H. Wang *et al.*, 2022)**. Tight junctions (TJs) between intestinal epithelial cells are crucial for maintaining cell polarity and regulating the permeability of the intestinal barrier **(Soderholm & Pedicord, 2019)**. TJ-related markers serve as reliable indicators of the extent of intestinal damage. The results of these experiments demonstrated that POS effectively alleviated the low expression levels of zonula occludens-1 (ZO-1), occludin, and claudin-1 induced by DSS **(H. Wang *et al.*, 2022)**. Furthermore, an additional experiment utilizing pectin and POS to generate POS *in vivo* produced comparable results. Moreover, researchers have reported that POS facilitates the recovery from DSS-induced colitis and enhances intestinal barrier functionality **(Zhang *et al.*, 2024)**. These findings suggest that POS therapy may aid in restoring intestinal barrier integrity while mitigating the inflammatory response within the gut. This histological evidence underscores the therapeutic benefits of POS in treating conditions such as colitis.

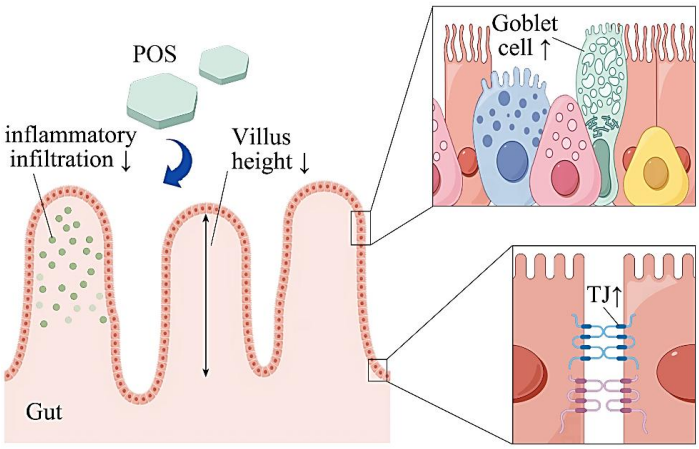

Figure 3 A schematic illustrating the potential effects of POS on intestinal micromorphology.
POS slightly reduced the intestinal villus height, increased the number of goblet cells, and significantly increased the weight ratio of the colon-to-intestinal contents. It also alleviated DSS-induced colonic shortening and weight loss, promoted intestinal mucosal repair, reduced inflammation, and increased tight junction protein expression.

POS DEMONSTRATES PROMISING ANTICANCER PROPERTIES

Numerous studies have explored the potential of POS to inhibit the proliferation of cancerous cells. Ho Jin Kang and colleagues were the first to report the antioxidant properties and the ability of irradiated citrus pectin POS to inhibit cancer cell proliferation **(Kang *et al.*, 2006)**. Subsequently, Sabeeta Kapoor demonstrated that POS derived from tomatoes exhibited anticancer properties in gastric cancer cell lines. These findings suggest that, unlike doxorubicin, which is cytotoxic to both cancerous and non-cancerous cells, POS showed no significant inhibition of normal cell proliferation while effectively eliminating cancerous cells. Furthermore, POS induced dose-dependent cell lysis and apoptosis in cancer cells, while not causing necrosis **(Kapoor & Dharmesh, 2017)**. Therefore, POS holds promise as a complementary anticancer therapy, providing additional options for cancer management.
Other studies have sought to elucidate the potential anticancer mechanisms of POS. An experiment utilizing swallow roots to extract POS demonstrated that it inhibited the proliferation of B16F10 cells (mouse melanoma cells) by blocking galectin-3 and survivin, while also promoting apoptosis **(Mallikarjuna & Dharmesh, 2018)**. Another study focused on POS's capacity to impede the development of bladder cancer, revealing that POS does not exert a cytotoxic effect on normal human cells but can inhibit the proliferation of T24 bladder cancer cells. These findings indicate that POS also reduces the migration and aggressiveness of bladder cancer cells. Annexin V-FITC/PI staining showed that POS increased the apoptotic rate and induced cell cycle arrest in the S phase, thereby triggering apoptosis. Regarding molecular pathways, POS was found to elevate the level of ST6Gal-1 and repress the expression of matrix metalloproteinases (MMP)-2, MMP-7, MMP-9, B-cell lymphoma (Bcl)-2, and cyclin D1. Conversely, POS was shown to stimulate the expression of Bcl-associated X protein 4 (Bax). The administration of POS reduced the levels of the zinc finger protein GLi1 (Gli1) and sonic hedgehog (Shh) proteins, which are crucial components of the hedgehog signaling pathway. In conclusion, animal experiments demonstrated that the growth of bladder cancer cells in the POS group was inhibited, and the expression levels of Gli1 and Shh were decreased, confirming the feasibility of the proposed pathway observed in cell experiments **(Huang & Wang, 2021)**. These experiments provide preliminary evidence of the anticancer potential of POS. However, the current experimental results are limited to a small number of cancer types, necessitating further comprehensive experimentation to elucidate the full range of cancers for which POS may serve as an effective anticancer agent.
Notably, the gut microbiota plays a significant role in cancer chemotherapy **(Guthrie & Kelly, 2019)**. It may influence drug metabolism, immune responses, and the production of metabolites, thereby enhancing the efficacy of chemotherapy and reducing its side effects **(Deng *et al.*, 2024; Li *et al.*, 2023; Matson *et al.*, 2021)**. Based on this experience, it can be postulated that modulating a patient's gut microbiota to increase the efficacy and mitigate the toxicity of chemotherapy drugs may represent a promising strategy in tumor therapy. The probiotic potential of POS makes it a suitable candidate for use as a chemoradiotherapy supplement in cancer treatment. It has been demonstrated that prebiotics based on various oligosaccharides exhibit comparable effects, such as galactooligosaccharides, fructooligosaccharides (FOS), arabinoxylan, β-glucan, and inulin **(Ziemons *et al.*, 2024)**. Chitosan oligosaccharides have also shown anticancer properties **(Azuma *et al.*, 2015)**. Pectin, as a macromolecular polymer of POS, is extensively employed in anticancer therapy and the development of novel anticancer drugs **(Carrion *et al.*, 2021; Emran *et al.*, 2022)**. In light of these findings, further research and exploration into POS as a potential anticancer prebiotic are warranted. However, it is essential to approach the anticancer potential of POS with considerable caution, especially when contrasted with its relatively well-established anti-inflammatory effects. The intricate nature of cancer pathophysiology, coupled with the severe consequences of the disease, presents significant challenges in translating POS into practical clinical applications. Therefore, it may be more prudent to regard POS as a potential adjunctive therapy in cancer treatment, rather than as a primary therapeutic modality, such as a chemotherapy agent.

CONCLUSION AND EXPECTATION

The probiotic activity of POS and its potential in anti-inflammatory immunity, lipid metabolism, maintenance of normal intestinal morphology, and anticancer properties provide strong evidence. These findings indicate that POS is a promising functional prebiotic food. Notably, the prebiotic activity of POS is primarily achieved through its regulation of the gut microbiota, which plays a key role in its therapeutic effects. Moreover, POS demonstrates drug-like biological properties, enabling it to target and regulate various biochemical processes. A substantial body of research has focused on the anti-inflammatory and immune-modulatory properties of POS, with the majority of studies examining its intestinal protective effects in the context of colitis. However, it is equally important to explore its potential in regulating lipid metabolism and its anticancer capabilities, areas that remain under-explored. The diverse biological activities of POS offer promising opportunities for the prevention and treatment of chronic diseases and metabolic syndrome, which continue to present major health challenges.
The existing research provides valuable insights into the mechanisms and biological targets of POS prebiotic activity, which can guide future investigations and inform potential applications. Nevertheless, most POS research is still at the laboratory stage, with many studies focused on qualitative exploration and feasibility rather than quantitative assessment of its biological activity and therapeutic effects. It is crucial to explore innovative combinations of current findings and consider future research directions. This could include more in-depth studies on the precise molecular mechanisms underlying POS's effects and its broader clinical applications. Furthermore, while POS shows promise as a prebiotic functional food, its use as a supplementary food or adjuvant therapy alongside drug treatment needs further investigation. Current research lacks sufficient data on the safety, dosage, and potential for multidrug interactions with POS, underscoring the need for further studies and clinical trials. These gaps limit the clinical application of POS and highlight the need for research to bridge the gap between laboratory findings and clinical implementation. Additionally, advancements in the preparation methods of POS are crucial for optimizing its production. While various POS preparation methods have been proposed in laboratory settings, there is a need for further discussion on the optimal pretreatment of raw materials and the development of scalable, industrial production processes for POS. We believe that future research on POS will provide new insights into the development and management of functional foods, paving the way for its eventual clinical use as a prebiotic functional food.

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