The Journal of Green Energy and Environmental Engineering

Review Paper

Functional Oligosaccharides: Research Progress in Preparation, Function, and Application

Tiamor Benedek [1], Irma Ruiz [1], Salvada Iranzo [2,*]

[1] Cátedra de Microbiología Agrícola. Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE. Ciudad Autónoma de Buenos Aires, Argentina
[2] Department of Microbiology, University of Valencia, Avda, Vicente Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain
*Corresponding author: Salvada.Iranzo@@uv.es

Abstract. Functional oligosaccharides are increasingly gaining public attention due to their various significant health benefits and have found numerous applications in areas such as health foods. However, the quantitative use and safety analysis of functional oligosaccharides still lack effective and extensive data support. The author, through methods including literature review, industry research, comparison of domestic and international regulations, and integration of health evidence with experimental results, summarizes the research progress of three common functional oligosaccharides regarding their sources, production processes, safety, dosage, and their application in health foods. The existing issues within the functional oligosaccharide industry are also discussed.

Keywords: *Functional oligosaccharides; preparation; functionality; application; irritable bowel syndrome*

Received on 02 July 2025, Accepted on 15 Oct 2025, Published on 15 Dec 2025

1 Introduction

Functional oligosaccharides are carbohydrates composed of 2 to 10 mono-sugars polymerized via glycosidic bonds, possessing various physiological activities [1]. Research has confirmed numerous health benefits, such as promoting intestinal health, enhancing immunity, and reducing serum cholesterol. Currently, the main functional oligosaccharides on the domestic market include: Fructo-oligo saccharides (FOSs), Galacto-oligo saccharides (GOSs), Xylo-oligo saccharides (XOSs), Isomalto-oligo saccharides (IOSs), Gentio-oligo saccharides, Lactosucrose, Lactulose, Soybean oligosaccharide, Stachyose, Raffinose, and over ten other types [2]. The types and sources of oligosaccharides added to existing health foods or commercial products vary. The source, processing technology, purity, component proportion in the product, product form (powder, liquid syrup, etc.), added amount, and recommended intake of different oligosaccharides can all affect the product's safety for consumption and the intended functionality. Therefore, studying the raw material consistency and product consistency of three functional oligosaccharides, clarifying the influence of raw material source, raw material production process, product purity, and product addition level on the safety and functional activity of the product, and establishing their dose-effect relationship are of great significance for establishing the raw material quality technical requirements and product quality technical requirements for these three functional oligosaccharides. The author elaborates on the research progress, safety, and use in the food industry of three functional oligosaccharides.

1.1 Sources of Oligosaccharides

Oligosaccharides are widely found in nature. Those relevant to human food mainly include: bananas, onions, barley, Jerusalem artichokes, etc. The types of oligosaccharides contained in these foods also differ; for example, Jerusalem artichoke contains fructo-oligosaccharides; yogurt contains galacto-oligosaccharides; bamboo shoots contain xylo-oligosaccharides; soybeans contain stachyose and raffinose; starch hydrolysates contain isomalto-oligosaccharides, etc. Japanese research has found that human milk also contains galacto-oligosaccharides.

1.2 Physicochemical Properties of Oligosaccharides

Oligosaccharides possess good physicochemical properties such as low calories, stability, safety, non-toxicity, and no residue; they also have important physiological functions like improving intestinal flora and enhancing immunity. The chemical essence of oligosaccharides is some short-chain carbohydrates that cannot be digested, generally small polymers formed by 2 to 10 monosaccharide units connected via glycosidic bonds, intermediate between monomeric monosaccharides and highly polymerized polysaccharides.

2 Physiological Functions of Oligosaccharides

Oligosaccharides can stimulate the proliferation of Lactobacillus in the rectum and enhance acetate production, thereby inhibiting the growth of putrefactive bacteria in the intestine, reducing the formation of toxic fermentation byproducts, improving metabolic functions, and promoting the absorption of mineral elements, among other beneficial effects. When the body is undernourished or during illness, the intestine often relatively lacks carbon sources that can be utilized by the gut microbiota, especially probiotics. After functional oligosaccharides enter the body, the metabolism of the gut microbiota undergoes significant changes.

2.1 Activating Bifidobacteria and Regulating Intestinal Flora Balance

Kohmoto et al. suggested that xylo-oligosaccharides, upon entering the intestine, can be utilized by beneficial bacteria in the gut, promoting the growth of beneficial microorganisms [1]. In vitro experimental results by Okazaki et al. showed that xylo-oligosaccharides significantly improve the intestinal flora [2]. Palframan et al. [3], Probert et al. [4], and Talwalkar et al. [5] suggested that after ingestion, functional oligosaccharides can selectively stimulate the proliferation of Bifidobacteria in the human intestine. These Bifidobacteria ferment functional oligosaccharides to produce large amounts of short-chain fatty acids and other metabolites, thereby ensuring the dominant position of Bifidobacteria in the host's intestine. Ingvar [6] and Xu et al. [7] suggested that functional oligosaccharides have excellent physiological effects as bifidogenic factors. Khaled [8] and Erica et al. [9] suggested that due to the unique physiological structure of functional oligosaccharides, various Bifidobacteria in the intestine can utilize different types of oligosaccharides. Knol et al.'s research showed that Bifidobacteria can proliferate extensively in the intestines of healthy humans by utilizing nutrients, forming a physiological barrier on the intestinal mucosal surface that directly resists the invasion of pathogens, such as common Salmonella typhi and Shigella dysenteriae [10]. Zhou et al. [11] used the B/E value as an indicator to conduct intestinal flora fermentation experiments in mice with common oligosaccharides on the market, including fructo-oligosaccharides, stachyose, xylo-oligosaccharides, and isomalto-oligosaccharides. Results illustrated that all four oligosaccharides enhanced the proliferation of Bifidobacteria and Lactobacilli, acidified intestinal pH, and benefited the intestinal microecological balance.

2.2 Lowering Intestinal pH and Inhibiting Intestinal Putrefaction

The biggest difference between functional oligosaccharides and ordinary oligosaccharides is that they are not digestible by the human body but can be utilized by beneficial bacteria in the human intestine, producing corresponding metabolites. Mihatsch indicated that upon reaching the intestine, functional oligosaccharides are fermented and utilized as an energy source by beneficial colonic bacteria such as Bifidobacteria and Lactobacilli, which are capable of fermenting these carbohydrates. This metabolic process generates substantial amounts of short-chain fatty acids (SCFAs)—primarily acetate, propionate, butyrate, and lactate—along with minor quantities of other byproducts. Lactic acid (LA), acetic acid (AA), and other SCFAs produced through the metabolism of beneficial bacteria can be absorbed by the small intestine and enter other metabolic pathways as energy substrates [12]. These SCFAs contribute to lowering the intestinal pH and elevating the intestinal osmotic pressure. The decrease in intestinal pH prevents the growth and reproduction of harmful bacteria, effectively reducing the generation of toxic and harmful intestinal putrefactive substances like formic acid, indole, and p-benzoic acid, and decreases the production and metabolic activity of harmful enzymes (such as β-glucuronidase), benefiting host health. Increasing the intestinal osmotic pressure allows the intestinal contents to absorb large amounts of water, thereby increasing stool volume and making it looser in structure, while also stimulating rapid intestinal peristalsis to promote bowel movements, preventing constipation. Zheng Jianxian's research results showed that xylo-oligosaccharides have a stool-softening function, mainly because intake of xylo-oligosaccharides increases the water content in the stool, thus changing stool morphology and preventing constipation [13].

2.3 Improving Lipid Metabolism and Promoting the Absorption of Mineral Elements

Many scholars have studied the effects of fructo-oligosaccharides and isomalto-oligosaccharides on the body's lipid metabolism. Shigeyuki et al. suggested that after ingestion of functional oligosaccharides, Bifidobacteria proliferate extensively and produce metabolites such as bile acid hydrolase. Bile acid hydrolase frees conjugated bile acids, and free bile acids can inhibit the growth of pathogens in the intestine [14]. With the extensive metabolism of probiotics, when the intestinal pH drops to 6.0, bile acids can combine with cholesterol and precipitate, being excreted from the body with the intestinal contents. Tasleem et al. suggested that the physiological effects of functional oligosaccharides largely depend on the products of their fermentation by the flora. Probiotics ferment functional oligosaccharides to produce organic acids, giving rise to a decrease in intestinal pH and an acidic intestinal environment, which causes complexes formed by calcium (Ca), phosphate (P), magnesium (Mg), etc., during passage through the small intestine to dissolve and be easily absorbed. Therefore, functional oligosaccharides can also promote the absorption of mineral elements such as calcium, magnesium, zinc, and iron [15]. Li et al. suggested that functional oligosaccharides themselves can also absorb cholesterol and be excreted with the stool. Thus, functional oligosaccharides have the effects of regulating blood lipids and lowering cholesterol [16]. Tan Yang et al. [17] and Wang Yu et al. [18] studied the laxative effect of mannan oligosaccharides on mice, and the results showed that mannan oligosaccharides have a good moisturizing and laxative effect on the intestines.

2.4 Other Physiological Functions

Coat et al. suggested that functional oligosaccharides can promote the proliferation of intestinal Bifidobacteria, which can produce various B vitamins, such as vitamin B1, B2, B6, B12, folic acid, and niacin [19]. Additionally, the decrease in intestinal pH can inhibit the growth of some vitamin-decomposing bacteria, thus ensuring that vitamin levels do not decline.

3 Progress in Extraction Technology of Oligosaccharides

Many methods developed for the extraction, fractionation, and purification of oligosaccharides domestically and internationally, mainly including five extraction and purification technologies: enzymatic, membrane separation, water bath, ultrasound-assisted, and microwave-assisted. Each technology has its own characteristics. Among them, microwave-assisted and ultrasound-assisted extraction have the shortest extraction time, high extraction rate, relatively simple equipment and extraction process, and can be applied to large-scale industrial production. Combining several methods may achieve better results.

3.1 Enzymatic Extraction Technology

Enzymatic extraction is a highly efficient extraction technology that uses specific and highly efficient enzymes such as cellulase, pectinase, and protease to disrupt plant cell walls and membranes, allowing active components to dissolve out quickly. Xu Guihua et al. used microwave and cellulase pretreatment of defatted soybean meal to extract soybean oligosaccharides. Results illustrated that after cellulase treatment, the extraction rate of soybean oligosaccharides increased by 15%–20%. The optimal conditions for enzymatic hydrolysis obtained through orthogonal experiments were: pH 3.5, temperature 45°C, enzyme addition 0.05%, enzymatic hydrolysis for 1 hour; the optimized conditions for alkaline extraction included: temperature 60 °C, pH 11, time 1.5 hours [20]. Song Zhaoxia used defatted soybean meal as raw material. Based on the optimal process conditions for extracting soybean oligosaccharides by alkaline extraction (extracting at 55°C and pH 11 for 1 hour), cellulase was used to pretreat the defatted soybean meal to investigate the effect of enzymatic extraction on the extraction rate of soybean oligosaccharides. After cellulase treatment, the extraction rate of oligosaccharides increased significantly [21].

3.2 Membrane Separation Technology

Membrane separation technology refers to the use of a semi-permeable membrane to retain colloidal-sized particles in water while allowing water and small solute molecules to pass through. Commonly employed membrane separation processes include ultrafiltration and nanofiltration. The underlying mechanism of ultrafiltration primarily involves mechanical sieving on the membrane surface, pore blockage, and adsorption on the membrane surface or within pores, with sieve filtration typically being the dominant mechanism. Gao et al. used ultrafiltration to study the extraction of soybean oligosaccharides and found that the ultrafiltration membrane used in the experiment had a relatively poor retention effect on oligosaccharides [22]. Dong et al. first used an ultrafiltration membrane to separate and purify Rehmannia glutinosa polysaccharides, and then used a nanofiltration membrane to concentrate and purify the ultrafiltrate. The optimal ultrafiltration conditions were: feed concentration 13–32 mg/mL, operating pressure 0.25–0.275 MPa, temperature 20–40°C, ultrafiltration twice. The suitable process conditions for concentrating and purifying the ultrafiltrate with the nanofiltration membrane were: feed temperature 20–40°C, operating pressure 0.59–0.79 MPa, and the concentration factor could reach 3 times [23]. Liu Qiao et al. studied the process of extracting soybean oligosaccharides from tofu wastewater using hollow fiber membrane ultrafiltration. The study showed that different pretreatment and ultrafiltration conditions resulted in different compositions of filtration resistance. Hollow fiber polysulfone membrane with a molecular weight cut-off of 10 kDa for ultrafiltration of tofu wastewater had a good effect on the separation of protein and oligosaccharides [24]. Ren Ye used nanofiltration separation technology to study the extraction of oligofructose with a molecular weight below 1500 from yacon. By investigating the initial material concentration, purification factor, and operating pressure, the nanofiltration operating conditions were optimized. The optimal conditions were: selecting a standard spiral-wound nanofiltration membrane element, initial material concentration 30 g/L, operating pressure 0.2 MPa, purification factor 15 times, which could achieve a monosaccharide removal rate of over 90%, and the content of oligofructose and inulin in the resulting product reached over 90% [25].

3.3 Water Bath

The water bath is the earliest traditional extraction method applied to the extraction of plant active ingredients. The basic process is to preprocess the fresh fruit, cut it into pieces and weigh them, then perform hot water stirring extraction under certain conditions (homogenization treatment), and finally centrifuge to extract the supernatant, which is the oligosaccharide extraction solution. The main influencing factors for extracting fruit oligosaccharides using traditional water bath extraction method include pH value, reaction time, temperature, and liquid-to-solid ratio. Wang et al. studied the process of extracting snow lotus fruit oligosaccharides using a homogenate assisted water bath method. When the homogenate speed was 2500 r/min, the homogenate time was 90 s, the extraction temperature was 80 ℃, the extraction time was 3 h, and the solid-liquid ratio was 1:20, the extraction rate of snow lotus fruit oligosaccharides was 63.2% [26]. In 2012, Ma et al. applied oligosaccharides yield as an indicator to investigate four influencing factors: alcohol concentration, extraction time, extraction times, and liquid to material ratio. Orthogonal experiments were used to optimize the hot water bath extraction process of oligosaccharides. The optimal extraction process obtained is: 50% ethanol extraction twice, liquid to material ratio of 6:1, extraction time of 2 hours per extraction, at which point the oligosaccharide extraction rate is 39.89% [27].

3.4 Ultrasound-Assisted Extraction Technology

Ultrasound-assisted extraction destroys plant cell walls through the unique mechanical vibration and cavitation effects of ultrasound on the medium inside the cells, allowing the solvent to quickly achieve into the plant cells, and the solute to rapidly dissolve and diffuse into the solution. Comparing with conventional extraction methods, ultrasound extraction can significantly reduce the extraction time and improve the extraction rate. Zhao Yingchun et al. used yacon as raw material and studied the extraction process of oligofructose using single-factor and orthogonal tests with ultrasound assistance. The optimal extraction parameters were: extraction time 3 hours, extraction temperature 85°C, ultrasound time 5 minutes, solid-to-liquid ratio 1:10. Under these conditions, the polysaccharide extraction rate was 4.257% [28]. Tian Yuting et al. used response surface methodology to study the effects of ultrasonic power, solid-to-liquid ratio, and extraction time on the yield of oligosaccharides extracted from lotus seeds with ultrasound assistance. The optimized parameters for ultrasound-assisted extraction of lotus seed oligosaccharides were: ultrasonic power 320 W, solid-to-liquid ratio

1:25, extraction time 48 minutes, and the oligosaccharide extraction yield was 1.13%. Compared with microwave-assisted extraction, the ultrasound-assisted method increased the yield of lotus seed oligosaccharides by 29.88% [29]. Tang et al. used bananas as raw material, studied the processes of extracting oligosaccharides by ultrasonic method and warm water method, and optimized the two extraction methods through orthogonal tests. The results showed that the optimized process for extracting banana oligosaccharides by ultrasonic method was: ultrasonic extraction time 40 minutes, ultrasonic power 500 W, solid-to-liquid ratio 1:2.5. Under these conditions, the yield of banana oligosaccharides was 17.89% [30].

3.5 Microwave-Assisted Extraction Technology

Microwave is a promising extraction technology. Its basic principle is: microwave radiation acts on the solvent and penetrates the cell wall to reach the interior of the cell. Because the polar solution within the cell absorbs microwave energy, the temperature rises sharply and internal pressure increases. When the pressure exceeds the bearing capacity of the cell wall, the cell wall ruptures, releasing the active components originally contained inside the cell, which then dissolve into the solvent. Through further filtration and separation purification, the desired extract can be obtained. Li et al. used microwave-assisted extraction to extract oligosaccharides from yacon. Using the extraction rate of yacon oligosaccharides as an indicator, they optimized the extraction process through orthogonal tests on six influencing factors: extraction temperature, extraction time, solid-to-liquid ratio, microwave power, microwave time, and resolving agent ratio. The obtained optimaized extraction conditions included: extraction temperature 80°C, extraction time 60 minutes, liquid-to-solid ratio 50:1, microwave power 700 W, microwave time 180 seconds, resolving agent ratio 7:1. Under these conditions, the oligosaccharide extraction rate reached 52.85% [31]. Zhou et al. studied the microwave-assisted extraction process of yam oligosaccharides. Based on single-factor tests, they conducted orthogonal tests on microwave power, ethanol concentration, solid-to-liquid ratio, and microwave treatment time. The order of factors affecting the extraction rate of yam oligosaccharides was: ethanol concentration > microwave treatment time > solid-to-liquid ratio > microwave power. The optimal conditions for microwave-assisted extraction of yam oligosaccharides were: microwave power 130 W, ethanol volume fraction 60%, solid-to-liquid ratio 1:30, microwave time 6 minutes. The extraction rate of yam oligosaccharides was 6.31% [32].

4 Physiological Functions of Individual Functional Oligosaccharides

4.1 Galacto-oligosaccharides (GOS)

Intestinal inflammation has emerged as a significant public health challenge in China. Inflammatory bowel disease (IBD) is a chronic intestinal inflammatory disorder triggered by an immune response following the disruption of intestinal homeostasis, typically resulting from impaired intestinal mucosal barrier function, increased mucosal permeability, and the entry of pro-inflammatory substances into the intestinal mucosa—a process involving factors such as the gut microbiota [1]. Recent studies indicate that the incidence of IBD in China is rising annually [2-3]. The etiology of IBD is complex, primarily associated with genetics, environment, immunity, and gut microbiota [2]. The intestine is not only the primary site for nutrient digestion and absorption but also an innate barrier that maintains the internal homeostasis of the body [11]. Intestinal homeostasis refers to the dynamic equilibrium established through interactions among the host (intestinal barrier), the intestinal microenvironment (including gut microbiota), nutrients, and metabolites [12]. Intestinal inflammation generally begins with damage to the intestinal barrier function, leading to disruption of intestinal homeostasis, which in turn causes acquired immune disorders, infiltration of neutrophils and macrophages, release of large amounts of cytokines and chemokines, increased expression of genes and proteins such as interleukin-6 (IL-6), cyclooxygenase-2, tumor necrosis factor-alpha in intestinal epithelial cells, and increased reactive oxygen species content, while decreasing the activity of catalase, superoxide dismutase, and glutathione peroxidase in the jejunum and ileum, inducing the development of intestinal inflammation and oxidative stress [13]. The intestinal barrier is primarily composed of intestinal epithelial cells (IECs) and tight junctions (TJs), among other components. It functions to prevent harmful intestinal substances (such as bacteria and toxins) from crossing the intestinal mucosa and entering systemic circulation, thereby defending against infection and inflammation [13-14]. Among them, IECs are the first line of defense of the intestinal barrier. Their development and health status (cell number, morphological structure) are not only positively correlated with nutrient absorption function [15] but also play a very important role in early immune defense and regulating intestinal barrier damage [16]. TJ proteins seal the gaps between IECs, forming an interwoven network on the intestinal lumen surface. They are an important structure for preventing luminal pathogens from passing through the paracellular pathway and a key factor in maintaining intestinal barrier function. TJ proteins are mainly composed of transmembrane proteins (Occludin, Claudin-1, etc.), cytoplasmic adhesion proteins (ZO-1, etc.) connected to the cytoskeleton. A decrease in the expression of TJ proteins caused by intestinal harmful substances and an increase in TJ permeability mean that the integrity of the intestinal barrier is compromised, and intestinal barrier function is dysregulated. This exacerbates oxidative damage or inflammatory responses, leading to acute or chronic intestinal inflammation [32]. Thus, ensuring intestinal homeostasis is of great significance for resisting the invasion of external substances and protecting human health.

GOS are the safest oligosaccharides and are a natural functional food additive [33]. In 2002, the U.S. Food and Drug Administration (FDA) listed GOS as one of the internationally recognized safe food additives [34]. GOS have been commercialized in Japan, the United States, Europe, and other regions. GOS are oligosaccharides formed by 2-9 galactose units and one terminal glucose connected by β-1,3, β-1,4, β-1,6 glycosidic bonds, using lactose as raw material and under the action of β-galactosidase (Figure 1) [6-9]. GOS have good water solubility, a sweetness of 0.3-0.6 times that of sucrose, are acid and heat resistant, and do not denature when kept at pH 3 and 160°C for 15 minutes. Research indicates that β-galactosidases derived from different sources directly influence the degree of polymerization, types of glycosidic bonds, and yield of galactooligosaccharides (GOS). Variations in the degree of polymerization and glycosidic bond types, in turn, determine the structure, activity, and prebiotic properties of GOS [36]. GOS connected by β-1,6 and β-1,3 glycosidic bonds exhibit strong prebiotic properties, possibly because GOS obtained from lactose hydrolysis and transglycosylation equilibrium reactions are more easily metabolized by probiotics themselves [36].

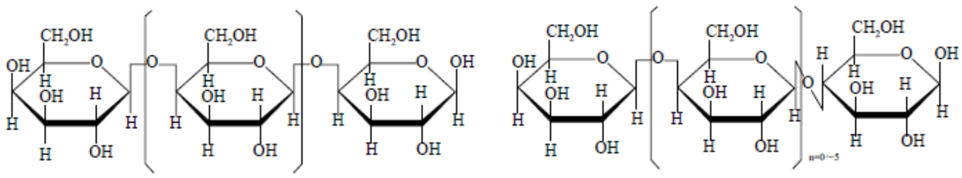

Figure 1 Molecular structural formula of GOS (left is the glucosyl group, right is the galactosyl group)

As an important prebiotic, GOS have various physiological functions, including: 1) maintaining intestinal flora balance and improving the intestinal environment; 2) improving the absorption of mineral elements (calcium and magnesium) [50], preventing osteopenia [51]; 3) regulating serum cholesterol content and improving lipid metabolism [32]; 4) preventing dental caries; 5) stimulating and enhancing immune response [35], anti-tumor; 6) inhibiting the expression of Toll-like receptor 2 in the brain of Alzheimer's disease model mice, reducing the M1-type activation of reactive astrocytes and microglia, reducing NF-κB p65 protein activation and pro-inflammatory cytokine expression, thereby alleviating neuroinflammatory responses and exerting anti-aging [36] and other physiological functions. There are extensive interactions between the gut microbiota and the host, which play a crucial role in maintaining intestinal homeostasis [37]. Galactooligosaccharides (GOS) can effectively ameliorate functional impairments in intestinal inflammation because the human gastrointestinal tract lacks the enzyme system required to hydrolyze GOS. As a result, GOS remain undigested and reach the colon, where they selectively stimulate the growth and proliferation of intestinal probiotics [38], inhibit the proliferation of harmful microorganisms and the production of putrefactive substances, and thereby help maintain the balance of the intestinal flora. Therefore, oral prebiotics may be more effective than oral active probiotics [39]. Due to immature gastrointestinal function, antibiotic use, and lack of breastfeeding, preterm infants have delayed colonization of gut microbiota and significantly reduced diversity, affecting the maturation of the intestinal and systemic immune system, and in severe cases, leading to necrotizing enterocolitis [40]. GOS can regulate the structure and proportion of the gut microbiota, repair intestinal barrier function, and alleviate intestinal inflammation. After adding GOS to infant formula milk powder, the number of Bifidobacteria and Lactobacilli and the acetate content in infant feces significantly increased [41], thereby promoting the development of the infant's immune system. GOS can intervene early in the gut microbiota of premature newborn rats, increasing the number of Bifidobacteria and reducing the number of Enterococci [42]. Early intervention with GOS can improve the morphological structure of the ileum in suckling piglets, enhance the digestion and absorption capacity of carbohydrates, promote ileal intestinal development, regulate the composition of the ileal intestinal flora, and to some extent improve the barrier function of the ileum [43-44]. Using GOS can effectively alleviate the reduction in Bifidobacteria abundance caused by amoxicillin treatment and is beneficial for the recovery of gut microbial metabolic activity after drug withdrawal [45]. GOS can also ensure intestinal barrier integrity and alleviate intestinal inflammation by promoting the expression of TJ proteins. GOS can not only enhance the cell's defense against bacteria or viruses by maintaining the balance of intestinal flora but also regulate the epithelial barrier function by inducing the differentiation of intestinal epithelial cells and repairing cell wounds [46]. GOS promotes the expression of TJ proteins, alleviating the damage to villus structure and intestinal barrier function in B6C3F1 mice caused by deoxynivalenol [47]. Supplementing GOS can effectively improve intestinal barrier dysfunction secondary to severe acute pancreatitis infection [48]. Short-chain fatty acids produced by the decomposition of GOS can not only inhibit the growth and reproduction of exogenous pathogens and inherent fungi in the intestine by lowering the intestinal pH, improving the population ratio of microorganisms in the intestine [49], but also significantly increase the number of immunoglobulin A and immunoglobulin M in the intestine by regulating the NF-κB and AMPK signaling pathways, thereby preventing rotavirus diarrhea in suckling mice [50]. They can also activate the short-chain fatty acid receptor 43 on the surface of T cells, thereby inhibiting the proliferation of colon tumor cells and the inflammatory response [51]. Oral administration of drinking water containing 10 g/100 mL GOS preparation can increase the number of anti-inflammatory bacteria and reduce the number of pro-inflammatory bacteria, thereby potentially preventing the occurrence and development of rectal cancer [52].

4.2 Xylo-oligosaccharides (XOS)

XOS are oligosaccharides formed by 2-7 xylose molecules connected by β-1,4 glycosidic bonds (Figure 2) [36], with disaccharides and trisaccharides being the main components [37]. Their physiological functions mainly include: regulating the balance of intestinal flora, promoting the proliferation of probiotics, and selectively promoting the proliferation of Bifidobacteria (mechanism: Bifidobacteria contain D-xylosidase, which can decompose XOS into xylose, further converting it into organic acids, providing a carbon source for the growth of Bifidobacteria), and high biological activity, their efficacy is 20 times that of other polymeric sugars [38]; promoting the absorption of minerals such as calcium and iron, and increasing the solubility of ions such as calcium and magnesium; Bifidobacteria have an immune-activating effect, can enhance human immunity, and resist aging (mechanism: inhibiting the growth of putrefactive bacteria, thereby reducing harmful substances such as hydrogen sulfide, ammonia, indole, and skatole in metabolites), while also accelerating the excretion of tumorigenic substances, thereby reducing the incidence of tumors; promoting the absorption of nutritional elements and improving nutrient utilization [39]; protecting liver function (mechanism: Bifidobacteria preparations can inhibit the proliferation of toxin-producing harmful bacteria, thus playing an auxiliary role in the treatment of liver diseases), and reducing the formation of toxic metabolites [36, 40] and other effects. Further studies have shown that XOS can reduce the number of Enterococci. The mechanism is that Enterococci cannot utilize XOS, while XOS, as a bifidogenic factor, promotes the proliferation of Bifidobacteria. As the dominant bacteria, Bifidobacteria produce large amounts of acid, lowering the pH, and also produce metabolites, all of which are unfavorable for the growth of harmful bacteria. In addition, XOS have a strong adsorption capacity for pathogens and can carry adsorbed pathogens out of the body, thereby inhibiting pathogens and diarrhea.

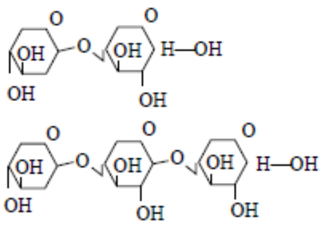

Figure 2 Chemical structure of Xylo-oligosaccharides (Xylobiose and Xylotriose)

4.3 Fructo-oligosaccharides (FOS)

FOS, also known as sucrose-derived oligosaccharides or oligofructose, have diverse structures and components. They are oligosaccharides formed by linking 1-3 fructose units to the fructose residue of a sucrose molecule via β-2,1 glycosidic bonds. Their composition mainly includes 1-kestose, nystose, and fructofuranosyl nystose. Natural extraction is relatively difficult, making it hard to form a certain scale. They have good moisture retention and hygroscopicity and stable pH [41]. Their main physiological functions include: promoting the proliferation of intestinal Bifidobacteria [42]; inhibiting the growth of pathogenic bacteria, changing the pH in the intestinal lumen, degrading pathogenic substances, thereby playing a role in expelling toxins and cleansing the intestine; enhancing human immunity and increasing the number of antibody cells; reducing cholesterol synthesis, improving lipid metabolism, preventing obesity; promoting the absorption of minerals such as calcium, iron, and magnesium [43] (mechanism: after FOS reach the large intestine, they are fermented and decomposed by Bifidobacteria, releasing mineral ions, which is beneficial for the absorption of mineral elements in the cecum and colon. Additionally, the decrease in pH is also conducive to the dissolution of minerals); as water-soluble dietary fiber with relatively low molecular weight, FOS can promote bowel movements and relieve constipation [44], among other important aspects.

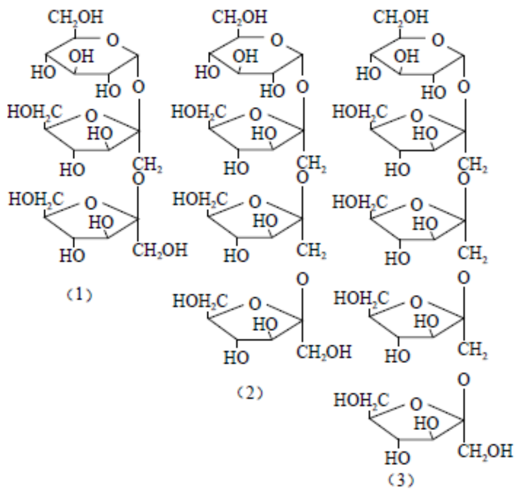

Figure 3 Structural formula of Fructo-oligosaccharides (FOS)

4.4 Isomalto-oligosaccharides (IMO)

IMO, also known as branched oligosaccharides, refer to oligosaccharides composed of 2-5 monosaccharide units where glucose molecules are connected by at least one α-1,6-glycosidic bond. They mainly include isomaltose, isomaltotriose, isomaltotetraose, and panose [37]. IMO syrup can give products a moist, fine, and soft taste and can undergo the Maillard reaction under certain conditions [38]. Their physiological functions mainly include: protecting liver function when liver function is abnormal [42]; promoting food digestion and absorption, maintaining normal intestinal function, improving gastrointestinal flora, and having a laxative effect [43] (mechanism: IMO promotes the proliferation of Bifidobacteria, which increase the secretion of propionic acid and butyric acid through sugar metabolism reactions. Organic acids promote intestinal peristalsis, while increasing fecal water content through osmotic pressure, thus having a laxative effect); synthesizing essential vitamins for the human body, promoting mineral absorption, regulating the metabolism of lipids in the body; enhancing immunity, preventing cancer and dental caries; being beneficial for the proliferation of Bifidobacteria in the intestine; improving serum lipids (mechanism: IMO proliferates beneficial bacteria such as Bifidobacteria, which in turn convert cholesterol into steroids not absorbed by the human body, thereby reducing cholesterol levels), preventing diarrhea, lowering blood pressure, and other important aspects [53].

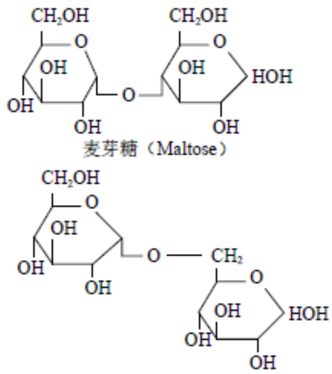

Figure 4 Structural formula of Maltose and Isomaltose

4.5 Galactomannan Oligosaccharides (GMOS)

GMOS are a general term for oligosaccharides composed of 2-10 galactose and mannose molecules polymerized through glycosidic bonds. They are a new type of functional prebiotic product. GMOS exist in endosperm tissue [46], and their raw materials include guar gum, locust bean gum, fenugreek gum, etc. [27, 47-48]. GMOS are incomplete degradation products of galactomannans, also known as galactomannan oligosaccharides. Their physiological functions mainly include: effects on intestinal microbial flora [51]; regulating nutrient metabolism, lowering blood sugar (mechanism: Galactomannan oligosaccharides (GMOS) are a type of water-soluble dietary fiber and share certain physiological functions with dietary fiber. They modulate lipid metabolism in the body through binding and adhesion mechanisms, thereby helping to lower blood lipids and cholesterol and improving glycemic responses. Additionally, GMOS can reduce the secretion of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) by the liver and decrease the activity and gene expression of fatty acid synthase. GMOS also promote the proliferation of beneficial bacteria such as Bifidobacteria. Both Bifidobacteria and Lactobacilli exhibit assimilation and co-precipitation effects on bile salts, which reduces the concentration of bile acids in the intestine. At the same time, the decrease in bile acid concentration leads to a decrease in the activity of lipase in the intestine, weakening the emulsification and decomposition of fat in food and reducing absorption); regulating intestinal function and improving constipation; enhancing immunity and antioxidant capacity; acting as feed additives with antibiotic functions, improving animal growth, fattening, and milk yield [52]. Furthermore, GMOS have a strong effect on scavenging DPPH free radicals [53].

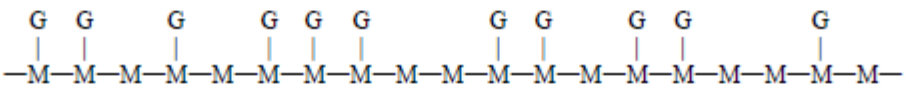

Figure 5 Structure of guar galactomannan

4.6 Soybean Oligosaccharides (SBOS)

SBOS are a general term for soluble oligosaccharides in soybeans, mainly composed of raffinose, stachyose, and sucrose [45]. SBOS have an obvious effect on inhibiting starch retrogradation and therefore can be used in starchy foods to extend the shelf life of food [4]; acting in the intestine of broilers, they can promote the proliferation of beneficial bacteria and inhibit the growth of harmful bacteria in the intestine of broilers (mechanism: SBOS can promote the proliferation of Bifidobacteria, which ferment oligosaccharides to produce short-chain fatty acids [acetic acid and lactic acid] and some antibiotics, thereby inhibiting the growth and reproduction of exogenous pathogenic bacteria and inherent putrefactive bacteria in the intestine), and reduce the ammonia content in feces [46]; Wang Jing et al. found in in vitro experiments that SBOS have a proliferative effect on lactic acid bacteria [47]; in addition, SBOS also have the following physiological functions: preventing constipation; preventing cancer (the anti-cancer effect is attributed to the cells, cell walls, and extracellular products of Bifidobacteria, which enhance the body's immunity); promoting the generation and absorption of nutrients; regulating fat metabolism; lowering blood pressure (some studies have shown that the level of human diastolic blood pressure is negatively correlated with the proportion of Bifidobacteria in the total bacteria in feces); protecting the liver; improving skin allergies, and other effects [45].

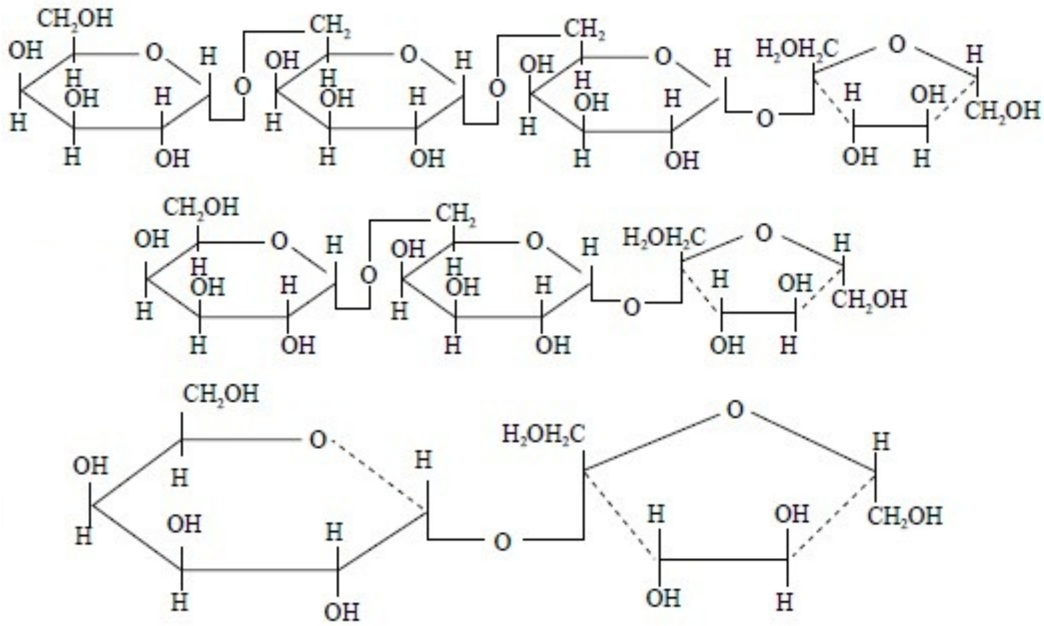

Figure 6 Structural formula of soybean oligosaccharides (SBOS)

5 Impact of Prebiotic Flour Products on Intestinal Health

Using flour products as a carrier, prebiotics are ingested by the human body and reach the digestive tract, where they regulate the intestinal microenvironment. The mechanism of action can be summarized into three aspects (Figure 7): regulating the structure of the intestinal flora, adjusting the types and levels of metabolites, and supplementing dietary fiber.

(1) Flour products act as carriers to deliver prebiotics into the body, where they are metabolized and utilized by the gut microbiota, meeting the complex nutritional needs of probiotics [26]. Microorganisms have selective differences in the utilization of prebiotics. The regulatory effect on the microbial community structure is primarily manifested by stimulating the extensive proliferation of probiotics such as Bifidobacteria and Lactobacilli, while inhibiting the growth of pathogenic bacteria including Escherichia coli, Clostridium, and Helicobacter pylori. Consequently, prebiotic flour products can alter the structure of the intestinal microbiota [27]. Walton et al. [28] observed that consumption of bread containing arabinoxylan oligosaccharides promoted an increase in fecal Bifidobacteria counts, along with a reduction in protein fermentation markers and an elevation in glycolytic fermentation end-products, indicating that arabinoxylan oligosaccharide-enriched bread promotes a beneficial shift in gut microbiota composition. Nissen et al. [29] reported that the addition of 4% olive pomace polyphenol fiber increased the fermentable protein-to-carbohydrate ratio in bread, making it more conducive to fiber fermentation than protein fermentation. Changes in bacterial populations in a multi-unit in vitro colon model also showed an increase in fiber-fermenting species (Bifidobacterium, Lactobacillus) and a decrease in proteolytic species harmful to humans (Enterobacter, E. coli, Clostridium) ($P < 0.05$).

(2) Promote the growth and activity of probiotics, adjust the types and levels of metabolites, among which the regulation of short-chain fatty acids (SCFAs) (mainly acetate, propionate, butyrate, and their salts) is the most concerning. After consuming prebiotic flour products, the level of SCFAs can be increased, thereby lowering the intestinal pH, acidifying the lumen, promoting the secretion of mucin, and providing a suitable colonization environment for probiotics. Costabile et al. [30] found that bread containing galacto-oligosaccharides

significantly promoted the increase of butyrate and acetate content, and could also inhibit the protein fermentation process harmful to host health, manifested as a decrease in the concentration of isovalerate, branched-chain fatty acids, valerate, and caproate. On the other hand, SCFAs can maintain 60%–70% of the energy supply for intestinal epithelial cells, help maintain intestinal barrier integrity, inhibit the invasion of pathogens on the surface of intestinal epithelial cells, and prevent inflammatory bowel disease (IBD). Lluansí et al. [31] found that patients with Crohn's disease and ulcerative colitis had increased diversity of gut microbiota and SCFA production after consuming three types of prebiotic bread fermented with Lactobacillus sanfranciscensis, providing a dietary treatment option for the potential way for IBD patients to restore intestinal health.

(3) Supplement the body with soluble dietary fiber. Prebiotics absorb water in the intestine, increase the volume of intestinal contents, stimulate intestinal peristalsis, accelerate intestinal transit, improve bowel function by increasing the water-holding capacity and volume of feces, acting as "intestinal scavengers," while buffering excess acid in the stomach and preventing constipation [32]. Hongisto et al. [33] found that fiber-rich rye bread shortened intestinal transit time, increased defecation frequency, softened stool consistency, and made defecation smoother, improving intestinal function in constipated women.

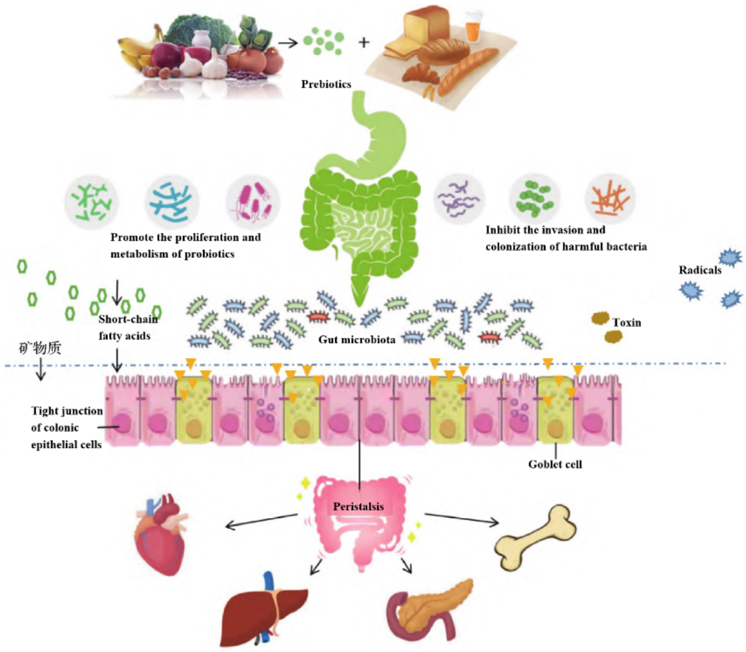

Figure 7 Relationship of prebiotic flour products and health

6 Applied Research on Functional Oligosaccharides

As novel physiologically active compounds, functional oligosaccharides have been extensively studied in areas such as nutritional health, animal husbandry, disease diagnosis and prevention, and plant growth and disease resistance. They have progressively evolved into a field that utilizes modern biotechnological approaches, including genetic engineering, protein engineering, and glycoengineering [48]. As ingredients for health-oriented foods, functional oligosaccharides were first widely recognized and commercialized in Japan and Europe. China started relatively late in the field of functional oligosaccharides but developed rapidly [49]. The following discusses the application of functional oligosaccharides in the fields of food, feed, and medicine.

6.1 Application of Functional Oligosaccharides in Food

The use of functional oligosaccharides in food products is the most common and widespread. They can be incorporated into various sectors such as bread, beverages, confectionery, biscuits, dairy products, condiments, noodles, infant formula, and frozen yogurt [50-53]. Shi Liang [44] investigated the application of

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oligosaccharides and probiotics in infant formula and found that adding oligosaccharides to infant formula helps maintain intestinal and immune health in infants. Meng Xianfang et al. [45] demonstrated that fructooligosaccharides (FOS) have a growth-promoting and protective effect on Lactobacillus acidophilus in frozen yogurt, increasing the viable bacterial count in the product. Yi Wenzhi et al. [46] studied the effects of four oligosaccharides—FOS, isomaltooligosaccharides (IMO), soybean oligosaccharides (SBOS), and xylooligosaccharides (XOS)—on the stability of brown beverages. The results showed that the composite use of the four oligosaccharides in a certain proportion had the best stability for brown probiotic milk beverages. Wang Xiaoqing et al. [47] studied the effects of FOS, stachyose, etc., on the proliferation of probiotics and the quality of yogurt, obtained the optimal process formula, and solved the taste problem of yogurt. Wang Yafang et al. [48] found that after human consumption of SBOS, the content of beneficial bacteria and short-chain fatty acids in the intestine increased. Yu Jiaojiao et al. [49] found that different concentrations of FOS and GOS had certain effects on the number of viable bacteria, titratable acidity, and viscosity of fermented milk during the fermentation and storage periods. Zhao Jin et al. [50] found that IMO applied to bread resulted in better product moisture retention, and added to ice cream, it was beneficial for improving its texture and taste. In addition, it can be added to beverages, wine, milk powder, yogurt, and other fermented products. The application of functional oligosaccharides in food has extended across diverse sectors. As functional health-promoting ingredients, they exert significant beneficial effects on human health (Figure 8) [15].

6.2 Application of Functional Oligosaccharides in Feed

The primary functions of oligosaccharides applied in feed include inhibiting pathogen colonization, promoting the proliferation of beneficial bacteria, protecting intestinal health in animals, enhancing immunity, and reducing the production of harmful gases such as ammonia in feces [51], among other effects. In feed, xylooligosaccharides (XOS), fructooligosaccharides (FOS), chitosan oligosaccharides, mannan oligosaccharides, soybean oligosaccharides (SBOS), etc., have been relatively well studied [52]. Zhou Zhenbing [53] pointed out that using oligosaccharides as feed additives in calf and pig feed can reduce the incidence of their gastrointestinal diseases and increase their growth rate. Wang Faming et al. [54] found that the application of functional oligosaccharides in piglet feed promoted the diversity of intestinal Lactobacilli in piglets. Hou Zhenping et al. [50] found that adding galactomannan oligosaccharides to piglet feed could increase the diversity of intestinal Lactobacilli in weaned piglets. Li Guiling [51] pointed out that adding galactomannan to dog feed also had a certain effect on the digestion of nutrients in dogs. The addition of functional oligosaccharides to poultry and livestock feed can modulate the balance of intestinal microbiota in animals, prevent diarrhea, and thereby enhance their growth rate. This field is currently experiencing rapid development and holds significant promise for future advancement.

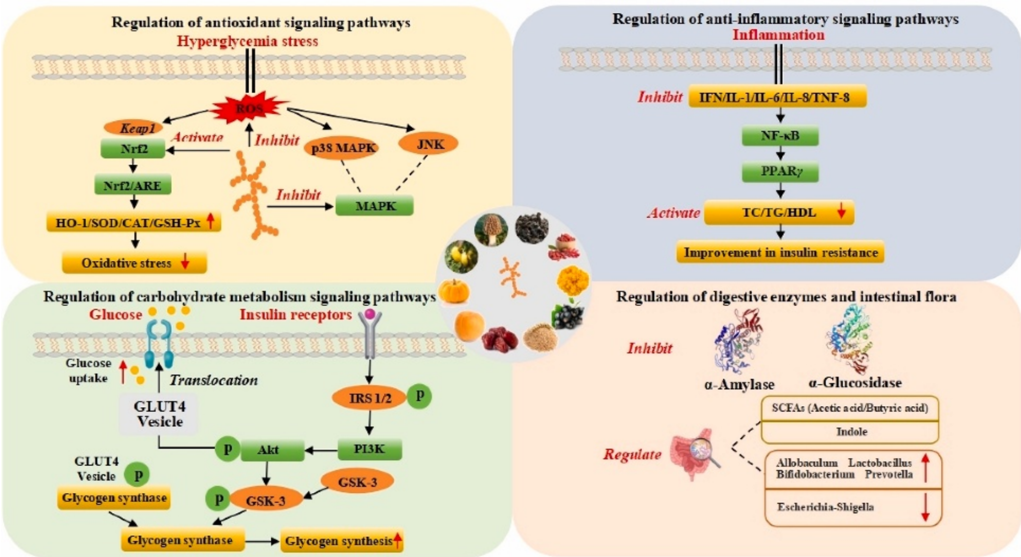

Figure 8 Potential hypoglycemic mechanisms of dietary polysaccharides.

6.3 Application of Functional Oligosaccharides in the Pharmaceutical Field

Oligosaccharides used in the pharmaceutical field mainly include chitosan. Chitosan can be used as a broad-spectrum antibacterial agent and also in tablets, granules, films, traditional Chinese medicine preparations, etc., and can inhibit the metastasis of cancer cells [54-56]. Recent research shows that galactomannan can also be used in the pharmaceutical field. Harshal A. Pawar et al. [8] found that galactomannan can be used in the preparation of captopril capsules. In addition, there are trehalose and xylitol, etc. Hou Lijun et al. [57] found in their study on trehalose that in addition to the properties of general oligosaccharides, trehalose has unique biological characteristics. It can not only protect organisms from oxidative stress damage but also protect them from hypoxia damage. It can be used to treat dry eye syndrome, Huntington's disease, reduce mucosal administration irritation, and protect cells, tissues, and organs. Zhang Songqing et al. [58] found that xylitol has adjuvant therapeutic effects in treating diabetes, liver disease, respiratory infections, etc.

7 Outlook

Prebiotics can effectively enhance the sensory quality and nutritional function of flour products, possessing broad development prospects and consumer markets. China has a good foundation in the development of prebiotic flour products. The following tasks urgently need to be carried out in the future: continue in-depth exploration of the actual nutritional functions of prebiotic flour products by combining in vivo and in vitro digestion experiments; explore the regulatory mechanisms of new prebiotics on gut microbiota at the molecular and metabolic levels; strengthen research on the interaction modes between prebiotic components and other components in flour products to improve the palatability, transportability, and stability of prebiotics; optimize the processing technology of prebiotic flour products to reduce the impact of anti-nutritional factors on the bioavailability of nutrients; conduct research on the synergistic effects of prebiotics with probiotics, postbiotics, and other functional components to provide a theoretical basis for the development of next-generation composite prebiotic flour products.

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