Food Chemistry: X 22 (2024) 101289

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Preparation, structural characterization, biological activity, and nutritional applications of oligosaccharides

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A R T I C L E I N F O

Keywords:
Functional oligosaccharides
Bioactivity
Food nutrition
Medicinal health

A B S T R A C T

Oligosaccharides are low-molecular-weight carbohydrates between monosaccharides and polysaccharides. They can be extracted directly from natural products by physicochemical methods or obtained by chemical synthesis or enzymatic reaction. Oligosaccharides have important physicochemical and physiological properties. Their research and production involve many disciplines such as medicine, chemical industry, and biology. Functional oligosaccharides, as an excellent functional food base, can be used as dietary fibre and prebiotics to enrich the diet; improve the microecology of the gut; exert antitumour, anti-inflammatory, antioxidant, and lipid-lowering properties. Therefore, the industrial applications of oligosaccharides have increased rapidly in the past few years. It has great prospects in the field of food and medicinal chemistry. This review summarized the preparation, structural features and biological activities of oligosaccharides, with particular emphasis on the application of functional oligosaccharides in the food industry and human nutritional health. It aims to inform further research and development of oligosaccharides and food chemistry.

1. Introduction

Oligosaccharides are usually found in nature in the form of glycoconjugates (glycoproteins or glycolipids), which are found in abundance in plants, animals, and microorganisms. They show a high structural diversity that greatly exceeds that of proteins (oligopeptides) and nucleic acids (oligonucleotides) (Weijers, Franssen, & Visser, 2008). Oligosaccharides have complex structures. As a biopolymer commonly found in living organisms, oligosaccharides are considered to play an important role in a variety of biological systems and signal recognition processes. It is the focus of attention in the field of life sciences.

Usually, they can be extracted from natural products. Homogenized samples of oligosaccharides and glycoconjugates can also be obtained chemically, enzymatically, or by other biological methods for systematic studies (Lv, Liu, Hao, Rahman, & Zhang, 2023) to translate human bioglycosylation into clinical applications (Cao et al., 2022). However, most oligosaccharides are not destroyed by the body's stomach acid or broken down by digestive enzymes, but they can be fermented and utilized by bacteria in the intestine to convert them into short-chain fatty acids and lactic acid. Oligosaccharides are of interest because they present important physicochemical and physiological properties that are beneficial to consumer health. Therefore, they and their derivatives have significant potential in the food industry to improve food quality and enhance food flavor.

In this paper, we first reviewed the research on oligosaccharides in terms of preparation sources, physicochemical properties, and bioactivity, and we furthered discussed the prebiotic activity of indigestible functional oligosaccharides. Herbal oligosaccharides play an important role in nutrition, health care, disease diagnosis, and prevention, having broad application prospects in various fields. We further briefly summarized the chemical composition and biological activities of several well-known herbal oligosaccharides. Finally, the paper reviewed the

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https://doi.org/10.1016/j.fochx.2024.101289
Received 11 October 2023; Received in revised form 11 March 2024; Accepted 12 March 2024
Available online 15 March 2024

application of oligosaccharides in the functional food market and the future development and innovation of non-digestible functional oligosaccharides in the food and pharmaceutical industry.

2. Definition and classification of oligosaccharides

Carbohydrates can be classified as monosaccharides, oligosaccharides or polysaccharides based on their molecular size or degree of polymerization (combination of the number of monosaccharide units). According to the definition of the Joint Committee on Biochemical Nomenclature, natural carbohydrates composed of 3–10 monosaccharide units consisting of linear or branched structures linked by α- or β-glycosidic bonds are defined as oligosaccharides(Mussatto & Mancilha, 2007), whose constituent monosaccharides can be glucose, fructose, galactose, and so on. However, many studies defined oligosaccharides as a general term for sugars whose molecular structure is usually formed by 2–10 monosaccharide molecules linked by glycosidic bonds (Rastall, 2010). Disaccharides (maltose, sucrose, lactose) are formed by the combination of two monosaccharides via a glycosidic bond (Qi & Tester, 2020). Two main types of covalent bonds connect them: the *N*-glycosidic type and the O-glycosidic bond. Polysaccharides are polymeric carbohydrates consisting of glycosidically bonded sugar chains with at least 10 monosaccharides, with molecular weights in the tens of thousands or even millions (Yu, Shen, Song, & Xie, 2018). Oligosaccharides have a molecular weight of 300–2000 and are the intermediate between monosaccharides (glucose, fructose, galactose) and polysaccharides (fiber, starch). The definitions of oligosaccharides differ in that one group of oligosaccharides includes disaccharides (i.e., carbohydrates with a degree of polymerization of 2), whereas the other group of oligosaccharides does not include disaccharides. More studies tend to define oligosaccharides as low-molecular-weight carbohydrates between monosaccharides and polysaccharides.

We generally divide oligosaccharides into two categories. One is oligomaltose, which is easy to digest, has low osmotic properties, and can extend the energy supply time to produce anti-fatigue function. After a long period of physical exertion or exercise, the human body is prone to dehydration, lack of energy reserves, and lowered blood glucose, and experiences impaired muscle neurotransmission, brain dysfunction, and a series of physiological changes and symptoms. The consumption of oligomaltose, can maintain blood glucose levels, and reduce the production of lactic acid, thus balancing insulin (Pan et al., 2017). The other type is isomaltooligosaccharide, which is considered a bifidogenic factor. After entering the colon, this kind of sugar can effectively promote the growth and reproduction of bifidobacteria, which is a beneficial bacterium in the human body. Long-term consumption of isomaltooligosaccharide can slow down the aging process and prevent cancer. Food containing this kind of oligosaccharide can play a nutritional and health care role in the human body (Goffin et al., 2011).

Carbohydrates can be classified as either digestible or indigestible based on their physiological properties. In recent years, much attention has been paid to functional oligosaccharides as food for specific health care purposes. Functional oligosaccharides are indigestible and are known as non-digestible oligosaccharides, which include the common ones such as fructooligosaccharides, cottonseed sugars, isomalto-oligosaccharides, lactulose, oligofructose, oligo xylose, oligogalactose, oligoisomaltose, oligoisomaltulose, oligoisomaltosucrose, oligogentiobiosaccharides, and soybean oligosaccharides. In recent years, new oligosaccharides such as marine oligosaccharides, human milk oligosaccharides (HMOs), and glycosaminoglycan oligosaccharides have attracted much attention. Marine oligosaccharides include chitin oligosaccharides, chitosan oligosaccharides, agar oligosaccharides, alginate oligosaccharides, and other oligosaccharides (Deng, Zhao, Zhao, & Zhao, 2023). Oligosaccharides of algal origin, which have complex branched chains and more reactive groups of marine organisms, have mostly higher biological activities than oligosaccharides of plant origin (Krishna Perumal et al., 2023). Glycosaminoglycans (GAGs) are involved in many key biological processes by regulating the activity of a variety of proteins, including growth factors, chemokines and viral receptors (Griffin & Hsieh-Wilson, 2013), GAG oligosaccharides can be divided into heparin oligosaccharide, hyaluronic acid oligosaccharide, and chondroitin sulphate oligosaccharide, among others. No enzyme in the human gastrointestinal tract can hydrolyze functional oligosaccharides. Therefore, they are not digested and absorbed but directly enter the large intestine preferentially to be used by bifidobacteria, serving as the proliferation factor of bifidobacteria.

Functional foods will be the food of the twenty-first century. Functional oligosaccharide is an excellent base material for functional food. The new type of oligosaccharide will be an important functional reinforcing agent for special nutritional food. Special nutritional products to meet the needs of different groups of people are the focus of the development of the food industry in the twenty-first century, with new oligosaccharides being an important class of functional enhancers for these special nutritional foods. At present, more than 10 kinds of new oligosaccharides are in commercial production worldwide, which are widely used in various functional health products and foods. The specific classification of sugar can be seen in Fig. 1.

3. Preparation of oligosaccharides

Oligosaccharides are receiving increasing attention as prebiotic functional food ingredients. They can be extracted from various natural products by physicochemical methods. Homogenized samples of oligosaccharides and glycoconjugates can be obtained by biological methods such as enzymes (Lv et al., 2023). Oligosaccharides can be obtained by physicochemical purification and isolation of polysaccharides that are widely distributed in plants and animals, but obtaining homogeneous oligosaccharide samples for further study is a more important challenge. For decades, researchers have been working to develop methods similar to general-purpose automated systems to synthesize oligosaccharides. Such an approach would revolutionize the role of oligosaccharides in biological systems. The main challenge in achieving automated synthesis is the lack of universal synthetic methods. Currently, the two main methods for obtaining homogeneous oligosaccharides and glycocouplings are chemical and enzymatic synthesis (Wen et al., 2018). Isolation of oligosaccharides from natural sources is difficult. Therefore, obtaining purified homogeneous oligosaccharides for research, especially for the development of carbohydrate-based methods, relies particularly on chemical and enzymatic synthesis of oligosaccharides. The comparison of different extraction methods of oligosaccharides is shown in Table 1.

3.1. Physical methods

Oligosaccharides have a plant origin. Physical methods are generally the preferred method to minimize the use of hazardous chemicals and their impact on the environment. The most common physical preparation methods are hot water extraction and extractive extraction. With a suitable solvent (water, alcohol, etc.) selected for extraction based on the properties and source of the desired oligosaccharides, the fraction containing oligosaccharides can be extracted from the natural product. Natural oligosaccharides can be obtained through concentration, purification lyophilisation, and other steps. Single physical extraction operations are often challenging, needing to be combined with other methods to obtain better hydrolysis (Krishna Perumal et al., 2023). Ultrasound-assisted extraction is highly efficient, with low energy requirements and low water consumption (Chemat, Zill, & Khan, 2011), and it is suitable for the extraction of thermally unstable compounds by optimizing the extraction conditions, type and concentration of solvent, extraction time, and ultrasound temperature (Jovanovic-Malinovska, Kuzmanova, & Winkelhausen, 2015). The concentration of extracted oligosaccharides from fruits and vegetables was increased from two-fold to four-fold by ultrasonic extraction. Microwave-assisted extraction

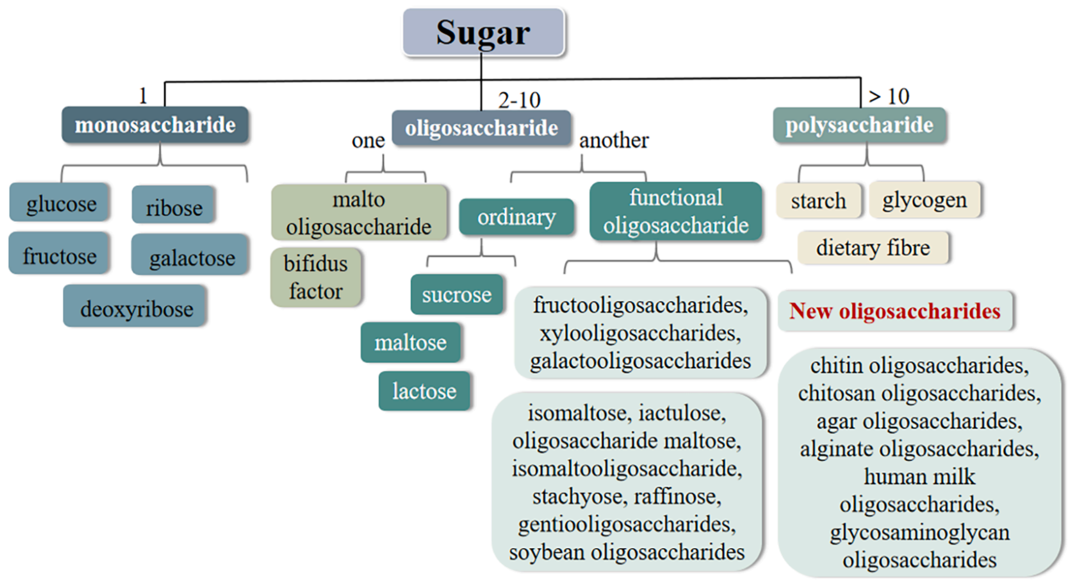

Fig. 1. Classification of sugars.

Table 1
Comparison of different extraction methods for oligosaccharides.

Method	Classification	Vantage	Drawback	Sphere of application	Bibliography
Physical method	Hot-water extraction	Easy to operate, safe and non-polluting	Inefficient and unable to produce on a large scale		(Lei et al., 2022)
	Distillation				
	Extraction	Controllable, adjustable	Extraction efficiency and yield issues		(Bordiga et al., 2019; Fu et al., 2021)
	Crystallization method				
	Membrane separation	High throughput, high yield	Difficulty in membrane Selection and optimisation	Obtain higher purity Oligosaccharides	(Basak & Annapure, 2022)
	Chromatography	Flexible and controllable	Take a long time		(Kruschitz & Nidetzky, 2020)
	Radiation law	Efficient extraction without affecting the active ingredients	High equipment requirements and safety risks		(Li, Liu, Zhou, Zhao, & Li, 2013)
	Ultrasonic method	High extraction rate, low energy consumption, short extraction time	Subject to ultrasonic attenuation coefficients		(Jovanovic-Malinovska et al., 2015)
Chemical process	Acid hydrolysis	High efficiency and wide range of applications	Not easy to control, causing environmental pollution	Preparation of oligofructose and xylooligosaccharide	(Liao et al., 2022; Lu et al., 2021)
	Alkali hydrolysis	Retention of active ingredients	Corrosive container, slower reaction rate		(Gao et al., 2014)
	Oxidation method	Polymerisation of oligosaccharides can be controlled	Destruction of structure by oxidising agents	Oligosaccharides with a low degree of polymerisation	(Xia, 2015)
	Chemical modification	Customised designs and modifications as required		Oligosaccharides with a low degree of polymerisation	(Lin, Zheng, Liang, Jiang, & Wei, 2021)
Biological method	Enzymatic	High selectivity, high yield	Poor stability and high cost	Most oligosaccharides	(Bujna et al., 2022; Gonçalves, Alves, Teixeira, & Nobre, 2023)
	Fermentation	Low cost, sustainable and suitable for mass production	long period	Highly selective for oligosaccharides	(Sun et al., 2020)
	Strain extraction method	Controllable, adjustable	Lower product purity	Highly selective for oligosaccharides	(Horlacher, Peist, & Boos, 1996)

showed superior operational performance compared with other conventional methods. Microwave-assisted heating instead of conventional heating can reduce the extraction time of chitosan, thus reducing energy consumption. Therefore, it is a more efficient and environmentally friendly method (Mohammadi et al., 2023).

3.2. Chemical process

Homogeneous oligosaccharide molecules obtained by chemical synthesis methods and their glycoconfixes formed with proteins or lipids have a broad spectrum of biological activities in anti-infective, anti-inflammatory, and antitumour functions. Chemical synthesis of oligosaccharide molecules plays an important role in exploratory studies of bioglycosylation (Lv et al., 2023). However, the chemical synthesis of oligosaccharides typically involves multiple functional groups linked by the formation of glycosidic bonds. Chemical synthesis involves tedious fine-tuning of the synthetic routes and stereochemistry, with the hydroxyl differentiation process being particularly difficult, which has made the conservation of groups and lengthy synthetic routes a hallmark of synthetic carbohydrate chemistry (Weijers et al., 2008). Moreover, the composition of products obtained through chemical degradation is always complex, which hinders subsequent separation and purification. At the same time, chemical degradation will cause a large amount of pollution (Li et al., 2007).

Chemical degradation is the classical method of polysaccharide depolymerization. Chemical modification of the polysaccharide molecule, such as the introduction of substituents and esterification reactions, produces oligosaccharides with a lower degree of polymerization. This method can be customized and modified as required. Acid–base degradation is one of the most common and effective methods to hydrolyze glycosidic bonds. In this method, the polysaccharide is heated under acidic or alkaline conditions to hydrolyze it to produce oligosaccharides. The process requires the use of strong acids or bases as catalysts. Oxidative degradation is one of the more studied degradation methods in the current chemical method. The most commonly used oxidant is hydrogen peroxide (Mao, Zhang, Sun, & Ren, 2012). The oxidation of hydrogen peroxide is related to the pH value of the solution. In neutral or alkaline solution, the reaction is mild, with few by-products, and it is a non-homogeneous degradation reaction. Under acidic conditions, the oxidation reaction is carried out in homogeneous phase, the reaction is fast, and the product with lower degree of polymerization can be prepared.

3.3. Biological methods

The glycosyltransferase and glycosidase families have great potential for the synthesis of biologically relevant carbohydrate structures. For the preparation of complex and high-purity oligosaccharides, methods based on the application of glycosyltransferases are currently regarded as the most efficient (Weijers et al., 2008). With the continuous development of enzyme engineering, multi-enzyme cascade catalysis technology has been applied to the development of functional oligosaccharides, thus promoting the green and sustainable development of functional oligosaccharide industry (Deng et al., 2023). Functional oligosaccharides with different structures and functions can be obtained using different functional oligosaccharide-degrading enzymes. For example, crude enzymes from probiotic bifidobacterium strains could synthesize novel oligogalactans with a high prebiotic potential (Bujna et al., 2022). Fucoidan sulfate oligosaccharides could be prepared to use the degrading enzyme from the marine bacterium *Flavobacteriaceae* CZ1127 (Yu et al., 2013). Chondroitin sulfate was used as an anti-osteoarthritic food supplement, and chondroitin sulfate oligosaccharides could be prepared by cleavage of chondroitin sulfate by the chondroitinase enzyme from strains of *Microbacterium spp*. (Zhang, Han, et al., 2023). Chitin deacetylase allows the preparation of defined chitosan oligosaccharides (Bonin, Sreekumar, Cord-Landwehr, & Moerschbacher, 2020).

Enzymatic methods are often limited by low enzyme activity, poor specificity and thermal stability, and difficulty in reuse, which must be overcome by enzymatic synthetic pathways for functional oligosaccharides. Various optimization strategies for the enzymatic synthesis of functional oligosaccharides are carried out to address the problems of different enzyme catalytic systems.

4. Structural characterization of oligosaccharides

Usually, biological activity is closely related to the chemical structure, including molecular weight, monosaccharide composition, glycosidic bond connection and position. Oligosaccharides show diverse structures. Their monosaccharide structural units are mainly five-carbon and hexose sugars, which are basically composed of six monosaccharides: glucose, fructose, galactose, xylose, arabinose and mannose. Structural analysis of oligosaccharides is an important basis for the study of their constitutive relationships, but the structural diversity and complexity of carbohydrate compounds limit the study of oligosaccharide activity. Understanding the structure of oligosaccharides is important for the development of novel bioactive substances with natural oligosaccharides.

During the production of oligosaccharides, unwanted impurities such as proteins and glucuronic acid may be present and can be separated by ethanol precipitation, activated carbon adsorption, dialysis, column chromatography (Mei et al., 2013), and membrane separation (Kazłowski, Pan, & Ko, 2015) to remove these substances and purify the oligosaccharides. The purity of oligosaccharides is one of the most important quality indicators for enhancing their biological effects and producing commercial products. Gel filtration chromatography can produce high-purity oligosaccharides with the same degree of polymerization, but it is expensive and difficult to produce on a large scale. Membrane separation is simple and inexpensive, but the purity of the obtained oligosaccharides is also low (Cheong, Qiu, Du, Liu, & Khan, 2018).

Physicochemical property analysis can observe the color, odor, taste, morphology, stability, and its solubility in water and organic solvents of the oligosaccharide sample. The sample is easily soluble in water, insoluble in organic solvents such as ether, and trichloromethane, and has good thermal stability. The Molisch reaction is positive, which indicates that the sample is a saccharide. The FeCl3 reaction, iodine-potassium iodide reaction, sulfuric acid-carbazole reaction, and bis(2-hydroxyboryl)urea reaction are negative, indicating that the sample contains no phenolics, starch, glyoxalates, peptides, and proteins. Structural characterization of oligosaccharides can be performed using various methods, such as nuclear magnetic resonance and infrared spectroscopy. The molecular weight can be analyzed by high performance liquid chromatography, high-performance gel-filtration chromatography, and high-pressure size exclusion chromatography, among others (Cui et al., 2023; Zhang, Zhuang, et al., 2022). Identification of the monosaccharide fraction of the product by characteristic retention times compared with standards and mass spectrometry data (Wang & Cheong, 2023). Fourier transform infrared spectroscopy is also an important characterization method for the study of structures and functional groups in oligosaccharides, allowing rapid and efficient analysis of samples (Tran et al., 2022). Commonly used instrumental analytical methods for the structural characterization of oligosaccharides are shown in Table 2.

5. Oligosaccharide bioactivity

Although the study of structure–activity relationships remains a central issue in research on the bioactivities of oligosaccharides, the focus of current research has shifted from the discovery of various bioactivities to the mechanism itself. Oligosaccharides have the ability to improve gut microecology; exert antitumour, anti-inflammatory, antioxidant, and hypolipidemic effects, improve immune function; protect gut flora; and exert other biological activities. New functional oligosaccharides such as marine oligosaccharides, HMOs, and glycosaminoglycan oligosaccharides have attracted much attention. Among them, chitosan is a functional oligosaccharide that is currently the subject of a greater research attention. Chitosan is a partially deacetylated product of chitin, which is a copolymer composed of β-(1 → 4)-2-acetamido-D-glucose and β-(1 → 4)-2-amino-D-glucose units. Its biological activities include antibacterial, antiviral, antitumour, antioxidant, immunomodulatory, and cholesterol-lowering effects (Liaqat & Eltem, 2018), and chitosan is currently used as a biodegradable, biocompatible and non-toxic polymers (Bakshi, Selvakumar, Kadirvelu, & Kumar, 2020).

The specific mechanism of biological activity of chitosan is illustrated in Fig. 2.

5.1. Antioxidant

Oligosaccharides have strong scavenging effects on hydroxyl radicals and superoxide anion radicals. Xylo-oligosaccharides have superior acid and heat stability whose bifidogenic function is 10–20 times higher than that of other oligosaccharides, thus being more suitable for functional foods (Brenelli, Figueiredo, Damasio, Franco, & Rabelo, 2020). Peanut shells, yams, and kelp are accessible natural sources of antioxidants and functional xyloglucans (Chen, Zhu, & Wu, 2015; Rico, Gullón, Alonso,

Table 2
Commonly used instrumental analytical methods for structural characterisation of oligosaccharides.

Classification	Method	Appliance	Bibliography
Analysis of physical and chemical properties	Odor, taste, morphology, stability, solubility Molisch reaction, $FeCl_3$ reaction, iodine-potassium iodide reaction, sulphuric acid-carbazole reaction, bis(carbamide) reaction	Tested for solubility in hot water, insoluble in organic solvents, free of phenolics, starch, glucuronic acid, peptides and proteins	
Purity and relative molecular mass	HPLC, HPGPC, HPGFC, HPSEC	Homogeneity and relative molecular weight determination	(Dávila, Gordobil, Labidi, & Gullón, 2016; Moreno, Montilla, Villamiel, Corzo, & Olano, 2014; Zha et al., 2022)
Analysis of monosaccharide composition	GC, GC–MS, LC-MS, HPLC, CZE-AD	Composition of monosaccharides, molar ratios, oligosaccharide sequence linkages	(Dong et al., 2007; Hu et al., 2022; Lei et al., 2022)
Ultraviolet spectral analysis	UV	Nucleic acid, peptide and protein determination	(Jalaludin & Kim, 2021)
Infrared spectral analysis	IR, FT-IR	Glycolic acid substitution	(İspirli et al., 2023; Lei et al., 2022)
Nuclear magnetic resonance analysis	NMR	Chemical shifts, coupling constants glycosides, (α- and β-) substitutions	(Cérantola et al., 2004)
X-ray diffraction analysis	XRD	Determination of the precise three-dimensional spatial structure	(Srivastava, Panwar, Prashanth, & Kapoor, 2017)

Note: High Performance Liquid Chromatography (HPLC), High-Performance Gel-Permeation Chromatography (HPGPC), High-Performance Gel Fil-tration Chromatography (HPGFC), High-Pressure Size Exclusion Chromatography (HPSEC), Ultraviolet Spectrophotometry (UV), Infrared Spectroscopy (IR), Fourier Transform Infrared Spectroscopy (FT-IR), Gas Chromatography (GC), Mass Spectrometry (MS) Gas Chromatography-Mass Spectrometry (GC–MS), Liquid Chromatograph-Mass Spectrometer (LC-MS), Nuclear Magnetic Resonance Spectroscopy (NMR), Capillary Zone Electrophoresis with Amperometric Detection (CZE-AD), X-ray Diffraction (XRD).

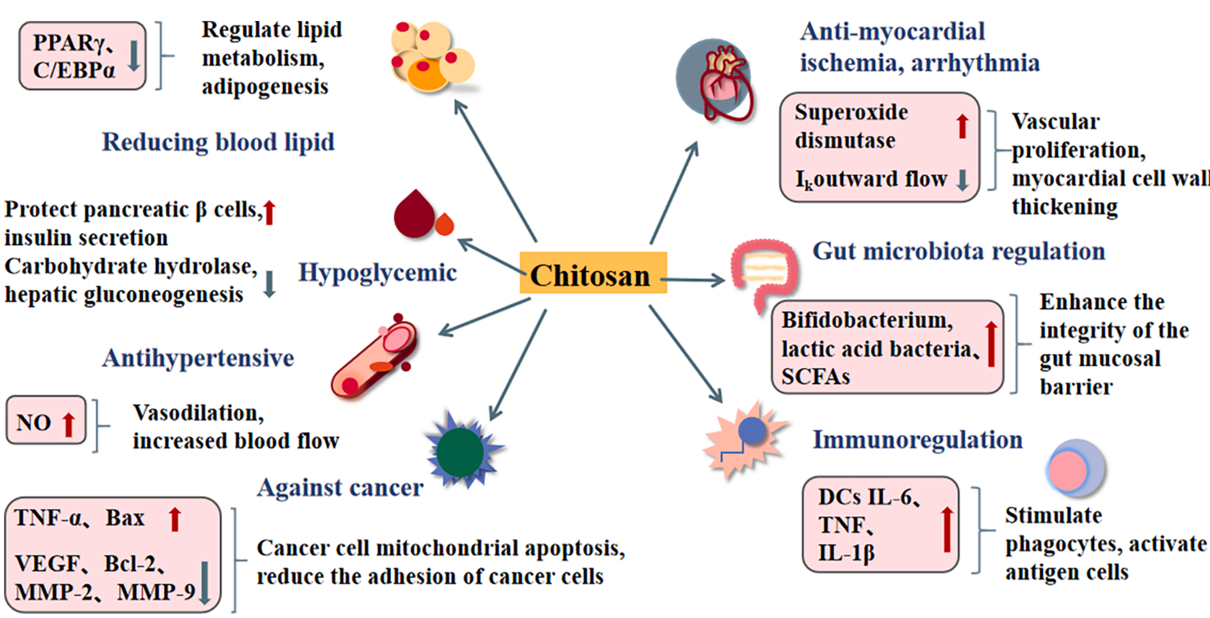

Fig. 2. Bioactivity mechanism of chitosan. Notes: peroxisome proliferator-activated receptor gamma (PPARγ), CCAAT enhancer-binding alpha protein (C/EBPα), interleukin-1β (IL-1β), interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-alpha), B-cell lymphoma (Bax), B-lymphoblastoma-2 gene (Bcl-2), matrix metalloproteinase (MMP) gene family, and short chain fatty acids (SCFAs).

Parajó, & Yáñez, 2018). Golden mushroom (*Flammulina velutipes*) oligosaccharides were hydrolyzed with hydrogen peroxide to obtain golden mushroom oligosaccharides, the results showed that they exhibited strong hydroxyl radical activity and reducing ability at a concentration of 100 μg/mL (Xia, 2015). In addition, agar oligosaccharides derived from marine products also exhibit interesting biologically active properties, including antioxidant, whitening, and moisturizing properties. Its antioxidant activity is often associated with anti-aging, which is why agar oligosaccharides are added to cosmetics as an anti-aging ingredient (Chen, Fu, Huang, Xu, & Gao, 2021). *N*-furfuryl chitosan (NF-CS) and *N*-furfuryl chitosan oligosaccharide (NF-COS) are prepared by acylation of chitosan and chitosan oligosaccharides, the results showed that they exerted the scavenging effect and reducing ability of DPPH in excess of chitosan as chitosan derivatives (Zhao, Wang, Tan, Sun, & Dong, 2013).

5.2. Anti-inflammatory

Inflammation is an innate immune response triggered by foreign antigens and injurious stimuli, and it is often accompanied by a variety of diseases such as diabetes, cancer, and atherosclerosis (Mao, Wang, Yue, & Xia, 2021). Severe acute inflammation may lead to tissue damage, sepsis, and death. Oligosaccharides express and inhibit the activation of inflammatory pathways, playing a role in the treatment of various inflammatory diseases such as acute colitis, steatohepatitis, and diabetic nephritis. Hyaluronic acid disaccharide could reduce lipopolysaccharide (LPS)-induced pro-inflammatory cytokine production in vivo. Its role as a natural metabolite of hyaluronic acid polysaccharides, whose acetyl group and conformation are determinants of inflammation, means that it may have a potential role in LPS-mediated inflammatory diseases (Han et al., 2022). In addition, oligosaccharides are fabricated into novel biomaterials and combined with drugs for targeted delivery to the site of inflammation. For example, vancomycin-loaded oligochitosan was made into nanoparticles. The structure showed that the additional interaction between vancomycin and oligochitosan helped enhance the antimicrobial and antibiofilm activity, successfully enabling the local treatment of osteomyelitis (Zhang, Chen, et al., 2022).

5.3. Antitumor and anticancer

Tumor is a major disease threatening human health. Cellular experiments demonstrated that chitosan oligosaccharides had the most significant antitumor activity against C33A cells. Their antitumor mechanism was related to oxidative stress, as well as activation of intrinsic mitochondrial apoptosis and autophagy signalling (Zhao et al., 2019). In addition, the development of safe and efficient antitumor drugs and their delivery systems is important to improve the efficacy of antitumor drugs. Cyclodextrins, which are cyclic oligosaccharides obtained by enzymatic hydrolysis of amylopectin with a special structure of external hydrophilicity and internal hydrophobicity, have been widely used in gene therapy, immune cell therapy, immune-targeted therapy, and chemotherapy. Combining therapeutic targeting with the tumor microenvironment can lead to the development of cyclodextrin-based host–guest complexes loaded with regorafenib for colorectal cancer treatment, which was shown to exert anti-tumor effects by inhibiting tumor cell proliferation and lesion neovascularization in the CT26 mouse model (Bai et al., 2021). Sachie et al. evaluated in a mouse model of colon tumors *N*-acetyl-d-glucosamine oligosaccharides (NACOS) and glucosamine oligosaccharides for their antitumor activity after oral administration, showing that both significantly inhibited tumor growth and exhibited significant apoptosis in tumor tissues. Therefore, NACOS and glucosamine oligosaccharides can be used as candidate functional antitumor foods (Masuda et al., 2014).

5.4. Antihyperlipidemic

Oligosaccharides are similar to water-soluble plant fibers, which can improve lipid metabolism, and reduce blood cholesterol and triglyceride levels. Chitosan oligosaccharides are beneficial in suppressing dyslipidemia and reducing the risk of atherosclerosis and hyperlipidemia. In a high-fat diet-induced hyperlipidemic rat model, it modulated the lipid levels of hyperlipidemic rats in a dose-dependent manner. It could upregulated the expression of genes related to cholesterol excretion (CYP7A1, LXRA, PPARα, LDLR), while the expression of cholesterol synthesis genes (HMGCR, SREBP2) was reduced (Yang et al., 2019).

5.5. Gut flora regulation

The human gut commensal microbiota and associated metabolites have long been recognized as facilitators of host health (Liu et al., 2023). The gut microbiota can be modulated in a beneficial manner to maintain, restore or improve host health (Lordan, Thapa, Ross, & Cotter, 2020). Functional oligosaccharides have important prebiotic properties for gut health through their fermentation in the gut. Their mechanism of action is mainly to promote the production of short-chain fatty acids and to regulate the gut microbiota (Zhang, Jin, et al., 2022). It can improve the microecological environment in the human body, favor the proliferation of bifidobacteria and other beneficial bacteria, inhibit the growth of gut spoilage bacteria, and prevent constipation. Dietary oligogalactans regulate the homeostasis of the senescent gut by promoting changes in microbiome composition and host gene expression, resulting in decreased gut permeability and increased mucus production (Arnold et al., 2021). In addition, prebiotic mannan oligosaccharides can remodel the gut microbiome and enhance the formation of the neuroprotective metabolite short-chain fatty acids (SCFA), which could translate into a novel microbiota-targeting approach for metabolic and neurodegenerative diseases (Liu, Xi, et al., 2021). A study introduced inulin-type oligofructose into a rat model of chronic unpredictable mild stress (CUMS) by intragastric force-feeding and examined its antidepressant effects through behavioral tests, gut morphology, and corticosterone levels. The results showed that oligofructose alleviated depressive-like behaviors, repaired gut epithelial damage, and reduced corticosterone levels in plasma and urine of the model rats. In particular, it promoted the abundance of bacteriophage cyanobacteria, which exhibited antidepressant-like properties (Chi et al., 2020).

HMOs, which are the third most abundant component of breast milk after lactose and lipids, are complex sugars with unique structural diversity. Their multiple beneficial functions are thought to be exerted through direct or indirect interactions with the gut microbiota (Zhang et al., 2021). 2′-fucosyllactose (2′-FL) is part of the fucosylated, while lacto-*N*-neotetraose (LNnT) is part of the non-fucosylated neutral HMOs. Infants have better assimilation and tolerance to 2′-FL and LNnT, which could enhance autoimmunity, influence cognitive domains, and improve learning and memory in rodents (Vázquez et al., 2015). HMOs affect not only infants but also adults 2′-FL and LNnT could alter gut microbiological composition and increased the number of bifidobacteria and actinomycetes (Elison et al., 2016).

6. Herbal oligosaccharides

Most herbal substances come from medicinal plants, with oligosaccharides receiving extensive attention at home and abroad because of their important biological activities and great medicinal potential. A large number of *in vitro* and in vivo experiments have shown that herbal-derived oligosaccharides such as Lycium barbarum, Astragalus membranaceus, acanthopanax, Panax ginseng, Morus alba, Angelica sinensis, and Ganoderma lucidum have a wide range of activities, such as antitumor, anti-inflammatory, and antioxidant properties; regulating gut flora; immune modulation; and hypoglycemic properties. Herbal oligosaccharides play an important role in nutrition, health care, and disease treatment, havimg a broad application prospect in the field of medicine (Liu, Cai, & Ding, 2021).

Currently, most functional oligosaccharides are synthetic, while oligosaccharides from natural plants, especially herbal oligosaccharides, are receiving increasing attention. For example, Ganoderma lucidum oligosaccharides have a good regulatory effect on gut flora, which could increase beneficial bacteria such as lactobacillus and bifidobacterium and reduce the number of harmful bacteria to regulate gut health. LBP-oligosaccharides are pyranose cyclic oligosaccharides with α-glycosidic and β-glycosidic bonds. As a potential source of prebiotics could restore gut and hepatic mitochondrial function, they can improve hepatic fibrosis in mice, regulat the structure and composition of the gut microbiota, and increase the proportion of beneficial bacteria. It also affects metabolites associated with cholesterol metabolism, bile acid metabolism and tryptophan metabolism pathways, effectively alleviating hepatic steatosis (Li, Zhang, Yu, Jia, & Cui, 2023; Zhang, Lu, et al., 2023). Radix ginseng oligosaccharides could improve anti-hypoxic activity by preventing lipid peroxidation and enhancing antioxidant activity (Xie et al., 2020). As an important immunomodulatory component, codonopsis oligosaccharides could increase the immune organ index, phagocytic index, and immunoglobulin content; stimulate the proliferation of splenic lymphocytes; and upregulate the expression of the corresponding mRANs and proteins. It could exert immunomodulatory effects through mitogen-activated protein kinases (MAPK) signaling pathway (Bai et al., 2020). Herbal oligosaccharide sources and specific biological activities are shown in Table 3.

Structural analysis of oligosaccharides is an important basis for the study of conformational relationships, and more in-depth studies that involve such analysis are needed. Traditional Chinese medicine oligosaccharides have a more complex chemical structure. However, the relationship between their various biological activities remains largely unclear and limits their potential future use. In addition, herbal oligosaccharides often have multiple potential targets. Their safe dosages

Table 3
Bioactive effects of herbal oligosaccharides.

Chinese herbal medicine	Source	Composition	Biological activity	Bibliography
Wolfberry *(Lycii Fructus)*	Solanaceae, Lycium barbarum	Glucose, fructose, mannose, rhamnose, consisting of 2–5 monosaccharide molecules	Antioxidant, anti-tumour, immunomodulation, hypoglycaemic maintenance of gut flora balance	(Jiang, 2014; Li et al., 2023)
Astragalus membranaceus *(Astragali Radix)*	Astragalus membranaceus, family Leguminosae	Glucose, galactose, composed of 2–6 monosaccharide molecules	Enhance immunity, antioxidant, anti-tumour, anti-inflammatory, blood sugar regulation	(Lim, Yu, Kim, & Chung, 2016)
Stinging sugar (*Alhagi pseudoalhagi* Desv.)	Sugar granules condensed from the secretion of camelina branches and leaves in the subfamily Pteridophyta, family Leguminosae.	Sucrose trisaccharide, 2–10 fructose units	Regulate gut flora, laxative, anti-cancer, regulate blood sugar	
Morinda officinalis (*Morinda officinalis* F. C. How)	Roots of the dicotyledonous plant medicine Rubiaceae.	Glucose, galactose, composed of 2–5 monosaccharide molecules	Anti-dementia, anti-depression, anti-oxidative stress, memory enhancement, immune enhancement, sexual function enhancement	(Cha et al., 2019; Chi et al., 2020)
Jerusalem artichoke (*Helianthus tuberosus* L.)	Perennial herbs of the genus Sunflower, family Asteraceae	Oligofructose, glucose, galactose, consisting of 2–10 monosaccharide molecules	Antioxidant, hypolipidemic, regulates gut flora, inhibits weight gain and fat storage	
American ginseng (*Panax quinquefolius* L.)	Ginseng perennials, family Cincinnabaraceae	Glucose, galactose, arabinose, consisting of 2–10 monosaccharide molecules	Antioxidant, hypoglycemic, hypolipidemic	
Codonopsis pilosula (*Codonopsis Radix*)	Dried root of Codonopsis pilosula, family Platycodonaceae	Glucose, galactose, arabinose, consisting of 2–10 monosaccharide molecules	Antioxidant, anti-aging, beauty	(Bai et al., 2022; Yue, Xiao, & Chen, 2023)
Panax ginseng (*Panax ginseng* C. A. Mey.)	Dry roots of Ginseng	Glucose, galactose, arabinose, consisting of 2–10 monosaccharide molecules	Antioxidant, hypolipidemic, hypoglycaemic, immunomodulatory	(Zhao, Wang, Liu, Lv, & Lu, 2020; Jiao et al., 2014)
Mulberry (*Morus alba* L.)	Dried shoots of Morus alba, family Moraceae	Oligofructose, glucose, galactose, fructose, xylose, consisting of 2–10 monosaccharide molecules	Anti-tumour, hypoglycaemic, anti-bacterial, protect gut flora	
Angelica sinensis (*Angelica sinensis* (Oliv.) Diels)	Angelica sinensis dry root	Glucose, isorhamnose, mannose, consisting of 2–10 monosaccharide molecules	Regulates blood circulation, anti-aging, promotes wound healing	(Hsu, Tsai, & Tsai, 2014)
Burdock (*Arctium lappa* L)	Root of Burdock (Arctium lappa), family Asteraceae	Inulin, Oligofructose	Immunomodulation, antioxidant, hypoglycaemic, promote lactic acid bacteria growth	(Moro & Clerici, 2021; Sun, Zhang, Guo, Yu, & Chen, 2013)
Ganoderma lucidum (*Leyss.* Ex Fr.)	Dried fruiting bodies of the fungus Ganoderma lucidum or Ganoderma lucidum, family Polyporaceae.	Glucose, galactose, mannose, consisting of 2–10 monosaccharide molecules	Immunomodulation, antioxidant, anti-inflammatory, regulation of gut flora	(Xia et al., 2022)

need to be regulated, and their metabolism in the human body is more complex. Therefore, the analyses of the structure of Chinese medicine oligosaccharides and studies on the special pharmacological mechanisms of Chinese medicine oligosaccharides should be strengthened. Research on protein histology and metabolomics of Chinese medicine oligosaccharides should also be conducted to elucidate its mechanism of action. A scientific evaluation system and modern medication guidelines need to be established, conducting comprehensive correlation analyses from multiple angles, levels, and aspects. Efficient and safe natural oligosaccharides can be developed and screened as dietary supplements (Liu, Cai, et al., 2021). Such work is the inevitable trend of the future.

7. Application of oligosaccharides in food nutrition

With the development of the economy, people's living standards continue to improve, but this condition comes with the rising incidence of diet-related hypertension, hyperlipidemia, diabetes mellitus, and other chronic diseases year by year. Replacing traditional food with functional foods is recommended to improve dietary structure and prevent diseases. This topic is a research hot spot in the global pharmaceutical, food, and nutrition sector. The major functional food markets in the world are the United States and Japan, followed by the Asia-Pacific and European markets, which are lucrative niche markets in food production and will grow globally, which were expected to reach USD304.5 billion by 2020 at a CAGR of 8.5 %. Functional beverages are the fastest-growing segment of the functional food market with dairy products also being a major volume growth area (Bogue, Collins, & Troy, 2017).

Prebiotics can enhance the survival, growth, metabolism and beneficial health activities of probiotics in the digestive system. The most known examples from diet are inactive food components that migrate to the colon and are selectively fermented, such as lactulose, oligogalactose, oligofructose, oligosaccharides, and inulin and their hydrolysates (Granato et al., 2020). The superiority of functional oligosaccharides is that they have the properties of certain sweetness and certain sugars, but also function as dietary vitamins to promote the propagation of beneficial gut bacteria, inhibit the growth of harmful bacteria, and improve gut microbial homeostasis. Indigestible functional oligosaccharides are widely used in the food industry, improving food flavor and quality, promoting food health, and prolonging the shelf life of functional beverages, dairy products, wine products, cereal feeds, and health care products, showing great potential for application.

The use of oligosaccharides in food nutrition and the role they play in the human body is shown in Fig. 3.

7.1. Improvement of food flavor

The higher molecular weight of oligosaccharides relative to monosaccharides makes them more viscous, thus being used in foods to improve texture. Oligosaccharides can also be used to change the freezing temperature of frozen foods and control the intensity of browning due to the Maillard reaction in thermally processed foods. Oligosaccharides have advantages over sucrose in terms of taste, stability, and solubility. Functional oligosaccharides are typically 0.3–0.6

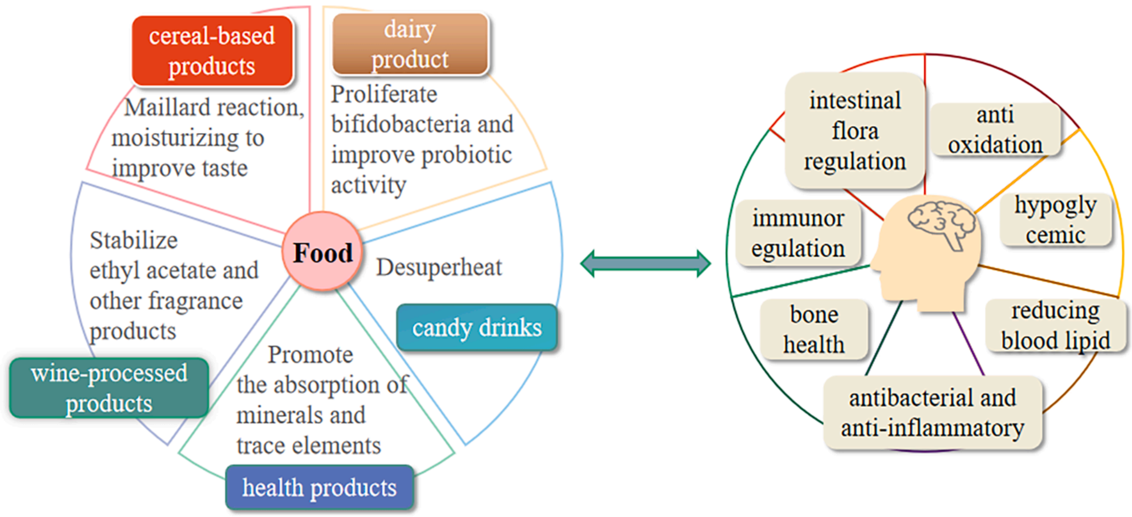

Fig. 3. Application of oligosaccharides in food nutrition.

times sweeter than sucrose. The sweetness depends on the chemical structure and usually decreases with the extension of oligosaccharide chain length (Roberfroid & Slavin, 2000). Some functional oligosaccharides such as oligoisomaltose, oligofructose, and oligolactofructose have some degree of sweetness and are good functional sweeteners. Oligosaccharides enhance caramelization during baking and do not affect the texture profile of baked items. As a result, caramelization increases, and baked products exhibit a darker color, crisper texture, and increased sweetness (Juhász, Penksza, & Sipos, 2020). Similarly, adding oligosaccharides to cheese products resulted in a more compact structure and an increase in apparent viscosity, elasticity, and hardness. Improved rheological and physicochemical properties (reduced viscosity and particle size, increased melting rate) and organoleptic properties (improved savory and sour taste, increased homogeneity, and reduced bitterness) (Ferrão et al., 2018).

7.2. Promoting food health

The use of sucrose is often limited by its high sweetness. Oligosaccharides are currently used in large amounts to replace sucrose in food flavorings. Because it is difficult to be hydrolyzed by salivary enzymes and small gut digestive enzymes, the calorific value is very low, rarely converted into fat. Oligosaccharides are non-insulin dependent, and will not raise blood sugar, thus being used in low-energy foods, such as diet food, and food for diabetic, hypertensive, and hyperlipidemic patients. Dietary alginate can be added to foods as a non-drug to regulate glucose levels in diabetics. It has been shown to be effective in regulating glucose metabolism and maintaining glucose homeostasis by ameliorating inflammation and apoptosis, decreasing postprandial insulin secretion, improving β-cell function, and normalizing lipid profiles (Chen & Gibney, 2023).

Oligogalactans promote the growth of beneficial bacteria, especially bifidobacteria and lactobacilli, thus maintaining a balanced gut microflora balance (Ambrogi et al., 2021). It is widely used as a functional food ingredient in prebiotic formulas and is a functional ingredient in infant formulas, thus attracting great interest from the infant nutrition industry (Ambrogi et al., 2023). Consumption of milk formulas enriched with oligogalactans, sometimes in combination with oligofructose, is currently recognized as a suitable alternative to breastfeeding. In addition, milk formulas enriched with HMOs are currently being researched and developed, but further scientific exploration is required to validate their presumed efficacy and specificity. Bifidobacterium-enriched HMOs degrade indigestible carbohydrates and subsequently produce beneficial metabolites (short-chain fatty acids, vitamins, and lactic acid) that shape the gut microbiota, produce antimicrobial effects, protect the gut barrier, and modulate the immune response. They are thought to play a favorable role, particularly in brain development (Moubareck, 2021). Oligogalactans are currently recognized for their safety in several countries, including the European Union, the United States of America, Japan, Australia, and New Zealand. Their preparations are used worldwide as bioactive ingredients in infant formulas. Although their use in infant nutrition is well established, the precise and long-term effects of oligogalactans on the subsequent development of the user still require considerable additional research. Given the variability and complexity of the carbohydrate composition of breast milk, combinations of oligogalactans with other prebiotics or synthetic HMOs to further improve infant formulas (Dinleyici, Barbieur, Dinleyici, & Vandenplas, 2023).

Some functional drinks, such as collagen drinks, which are enriched with collagen, contain prebiotics, dietary fiber, probiotics, and vitamins, regulating the microecology of the gut tract to achieve internal and external balance. Supplementation of collagen increases skin elasticity and reduces wrinkles. Prebiotics can quickly eliminate toxins and effects of a hangover in the gut tract. For example, coffee oligosaccharides have important physical, chemical, and physiological characteristics. They can be used as prebiotics and antioxidant dietary fibers in adjuvants, drugs, nutritional foods, intestinal health, immune system enhancement, cancer treatment and so on (Tripathi & Murthy, 2023). Inulin-type fructans (ITF), including short-chain fructooligosaccharides (scFOS), oligofructose, and inulin, are commonly used fibers that are widely regarded as prebiotics for their ability to be selectively utilized by the intestinal microbiota to confer a health benefits (Hughes, Alvarado, Swanson, & Holscher, 2022).

7.3. Improveing food quality to extend shelf life

Functional oligosaccharides play an important role in food preservation due to their antimicrobial activity. The heating reaction between chitosan or its derivatives and sugars or proteins improves their water solubility, effectively inhibits microbial growth, reduces lipid oxidation, improves organoleptic properties, prolongs shelf life, and enhances the quality of raw food (Hafsa et al., 2021). As an antimicrobial agent, it can effectively extend the shelf life of fresh wet noodles during storage at 4 °C and inhibit the increase in acidity value (Chen et al., 2023). Adding chitosan to ice cream increases hardness and decreases the melting rate, resulting in a more stable ice cream product.

7.4. Other applications

With the advocacy and promotion of green farming, as well as the requirements and regulations on the restricted use of antibiotics and other drugs, oligosaccharides will be increasingly used in animal health products and the feed industry. Indigestible oligosaccharides cannot be digested by enzymes in the small intestine of mammals, but can be fermented by bacteria in the hindgut of non-ruminants. Inulin, oligofructose, and oligogalactose can be added as prebiotics to pet food or feeds to reduce animal fecal odor and improve growth performance (Flickinger & Fahey, 2002). Dietary lactose and lactose intolerance are hot topics in the food and nutrition field today. Prebiotics and probiotics have been proposed as an alternative to avoid some of the symptoms of lactose intolerance (Szilagyi & Ishayek, 2018).

7.5. Safe dosage of oligosaccharides

Oligosaccharides are widely used in food supply because of their beneficial effects on human health and product development, but excessive intake can lead to adverse gastrointestinal side effects. Therefore, guidelines need to be set with regard to tolerable intake levels of different oligosaccharides (Mysonhimer & Holscher, 2022).The laws and regulations on oligosaccharides issued by the China General Administration of Quality Supervision, Inspection and Quarantine (CNCA), European Food Safety Agency (EFSA), Food and Drug Administration (FDA), and Food Standards Agency (FSA), set clear dose requirements for fructooligosaccharides and galactooligosaccharides because of their wide application, especially in infant food.

Oligofructose at 10–15 g/d has been shown to improve mineral absorption. Adult males with a single intake of less than 17 g, and females with a single intake of less than 14 g have no diarrhea. When the intake reaches 30 g/d, the subjects begin to experience gastrointestinal flatulence. Intake greater than 50 g/d causes abdominal colic or diarrhea (Briet et al., 1995). The amount of fructooligosaccharides used in approved health foods ranges from 4 g/d to 30 g/d. In addition, China allows fructooligosaccharides to be used in infant and maternal milk powder, but the total amount should not exceed 6.5 %, and infants can tolerate 4.2 g of fructooligosaccharides per day. The FDA also pointed out that the intake of fructooligosaccharides in the general population is up to 20 g/d. At high doses of fructooligosaccharides are taken, abdominal pain, diarrhea, and increased intestinal inflammation occur in animals and humans. For galactooligosaccharides, no clear dosage regulation is established for their use in general adult foods in various countries, but the addition amount is specifically limited in infant formula foods. The content of galactooligosaccharides in infant milk powder should usually be less than that in breast milk. The FDA lists galactooligosaccharides as an internationally recognized safe food additive, and the amount of galactooligosaccharides added in most foods is 0.5 %–6.0 %. Chronic (2–16 weeks) galactooligosaccharides studies reported a dose range of 2.5–20 g/d (Davis, Martínez, Walter, & Hutkins, 2010; van den Heuvel, Schoterman, & Muijs, 2000). However, good tolerance was also observed for galactooligosaccharides up to 20 g/d, this dose was shown to improve calcium absorption. For most adults, regular consumption of a small amount of oligosaccharide products rarely causes adverse symptoms, but may cause mild symptoms in children.

The source of oligosaccharide raw materials is not uniform and the purity of the product is not clear, thus, the evaluation of the functional indicators of the product is affected. There are differences in the requirements of laws, regulations, and standards in different countries vary, resulting in uneven recommended doses of oligosaccharides. The description of the applicable population of oligosaccharides in most national regulations is also very vague, especially for infants and children. The use requirements require more attention. At present, research on oligosaccharides tends to involve human and animal experiments, and few mechanistic studies such as cell experiments are conducted. Future research should also adopt a standard scheme, which can develop consistent search terms through advanced network technology, provide complete characterization of oligosaccharides, and summarize various evaluation reports and experimental data to clarify the mechanism of action, product purity, and corresponding recommended dosage of oligosaccharides.

8. Outlook

Oligosaccharides, as functional bioactive molecules, are an effective source of functional foods and nutraceuticals. They are used as functional food materials or incorporated into food matrices to improve the technical and organoleptic properties of foods. With the development of society and economic development, people's quality of life has been improved, and health consciousness gradually improved. The development of functional oligosaccharide food development has become a hot spot, and market prospects are extremely broad. Therefore, in-depth research on functional oligosaccharide is particularly important.

Structural analysis is an important basis for the study of conformational relationships, but the structural diversity and complexity of glycoconjugates limit the study of oligosaccharide activity. Understanding the structure and biological functions of oligosaccharides is important for the development of new bioactive substances using natural oligosaccharides. In particular, the relative structure of oligosaccharides from natural resources, especially marine algal bio-oligosaccharides, are more diverse and should be studied further. In addition, the quality control, resource exploitation and utilization of herbal oligosaccharides, and the mechanism of active ingredient synthesis have not been well studied. Detailed studies on its phytochemistry, pharmacology and molecular biology at a single level are urgently needed.

The main limitation and challenge in the current production of novel oligosaccharides is the lack of advances in extraction and characterization techniques. The differences in the bioactivity mechanisms of oligosaccharides prepared using different preparation techniques need to be fully investigated. The development of composite oligosaccharide products with unique physiological activities and diverse structures and their green synthesis technology will be an important research direction for the synthesis of functional oligosaccharides in the future. However, the key challenge to break through the efficient preparation of functional oligosaccharides lies in how to establish a systematic platform that can achieve precise extraction and characterization. With the rapid development of high-throughput technology screening, multi-omics sequencing technology, and protein computational design technology in recent years, the design of the pathway for functional oligosaccharide synthesis will develop in the direction of intelligence and high efficiency. It should be combined with artificial intelligence technology to develop new methods to optimize the preparation of oligosaccharides and to perform in-depth exploration of a variety of methods to study the role of functional oligosaccharides. With focus on the identification and modification of new specific enzymes with high stability and target specificity, the use of artificial intelligence to design new functional oligosaccharides with some special functions of specific enzymes may be a promising field for the production of oligosaccharides. Thus, strengthening the efforts of basic research to finally achieve an important breakthrough in oligosaccharide research is all the more important.

CRediT authorship contribution statement

Ya-jing Chen: Writing – original draft. **Xin Sui:** Writing – review & editing. **Yue Wang:** Supervision. **Zhi-hui Zhao:** Supervision. **Tao-hong Han:** Supervision. **Yi-jun Liu:** Validation. **Jia-ning Zhang:** Validation. **Ping Zhou:** Validation. **Ke Yang:** Funding acquisition. **Zhi-hong Ye:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Yang Ke reports financial support was provided by Ke.

Data availability

The authors are unable or have chosen not to specify which data has been used.

Acknowledgments

This work was supported by the key project at central government level: The ability establishment of sustainable use for valuable The National Key R&D Program of China under Grant (NO. 2021YFF0600804); The National Science Foundation of China (No. 82101714); China Postdoctoral Science Foundation (No. 2021M690259).

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