Antioxidant Activities of Natural Polysaccharides and their Derivatives

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Natural polysaccharides are a type of natural biomacromolecule found in plants, fungi, algae, animals, and bacteria. Due to their nontoxic, stable, biodegradable, biocompatibility, and excellent antioxidant activity, natural polysaccharides contribute to the potential value in treating or preventing disease caused by oxidative stress. Polysaccharides can reduce the damage to the cell structure, regulate the signal pathways related to antioxidation, improve the intracellular antioxidant enzyme system, reduce the substances that easily produce reactive oxygen species (ROS), and protect the body tissue from ROS-induced damage through free radical scavenging activity and immunomodulatory activity. Natural polysaccharides play an irreplaceable therapeutic role and have received more and more attention. Publications related to natural polysaccharides are also increasing.

natural polysaccharides | antioxidant | reactive oxygen species (ROS) | drug delivery

1. Antioxidant Activity of Natural Polysaccharides

1.1. Higher Plant Polysaccharides

Higher plant polysaccharides have the highest proportion of natural polysaccharides. They are also the type of natural polysaccharide with the most abundant sources, convenient extraction, and extensive research. It has been reported that plant polysaccharides have many pharmacological effects, such as anti-tumor and anti-diabetes effects. These pharmacological effects are closely related to antioxidant function.

Lycium barbarum polysaccharides (LBP) are the most bioactive polymer in *Lycium barbarum* fruit extracts and are widely used for food and medical treatment. Due to its multiple pharmacological effects, such as antioxidant, anti-cancer, anti-diabetes, and neuroprotection, LBP has been extensively studied in various disease models. The content of LBP is an important factor affecting the efficacy of *Lycium barbarum* [1]. Studies have shown that LBP can play an antioxidant role by removing superoxide anions from mitochondria in cells through an oxidation reaction. In addition, four molecular weight polysaccharide components were extracted from LBP using an integrated tandem hybrid membrane technology, and all of them have antioxidant activity. Ferric-reducing antioxidant power (FRAP) assay showed that the lower molecular weight polysaccharide component (LBP4 MW = 38 kDa) has more significant antioxidant activity. Moreover, the increase in polysaccharide concentration is also helpful to scavenge superoxide free radicals. As such, the four polysaccharides have scavenging activity that is maximal at 2.0 mg/mL [2]. LBP also indirectly acts on free radicals by capturing reactive oxygen species (ROS) produced during the peroxidative chain reaction. The polysaccharides capture ROS to reduce the level of lipid peroxidation and alleviate oxidative damage [3].

Some researchers have made the first study on the antioxidant activity of *Astragalus* polysaccharides (AP). The results showed that APs contain mannose, glucose, arabinose, and galactose, and their proportions decreased in order. Compared with normal rats, AP-treated rats could significantly inhibit the production of malondialdehyde (MDA) and increase superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) levels in the blood and liver [4]. Many factors affect the antioxidant activity of polysaccharides, such as the extraction method. Some researchers have evaluated the effect of extraction methods on the antioxidant activity of APs. By comparing hot water extraction, ultrasonic-assisted extraction, enzyme-assisted extraction, and enzyme ultrasonic-assisted extraction, some researchers found that the polysaccharides extracted by enzyme-assisted extraction had the highest extraction rate and antioxidant activity [5]. Recently, it was found that APs can reduce the side effects of tilmicosin in rats, mainly because APs can relieve tilmicosin-induced oxidative stress by causing a significant decline in the levels of MDA and other oxidative stress indexes [6].

Dendrobium officinale is a type of precious traditional Chinese medicine that has a remarkable curative effect on treating pharynx and lung diseases. In terms of pharmacological effects, flavonoids are found to have vital antioxidant activities [7]. In addition to flavonoids, *Dendrobium officinale* polysaccharides (DOP) also have antioxidant activity. Some studies found DOPs with a smaller molecular weight have more potent antioxidant activity through antioxidant tests such as DPPH radical scavenging, ABTS radical scavenging, and hydroxyl radical scavenging [8]. In addition to the influence of molecular weight, DOP obtained by the freeze-drying method (FD) has the best antioxidant effect [9]. Some researchers purified the crude DOP by chromatographic separation. It was concluded that the main monosaccharide components in the purified polysaccharides were mannose and glucose, which protect RAW 264.7 macrophages by resisting H_2O_2-induced oxidative damage [10].

As a well-known blood-tonifying medicine, *Angelica sinensis* has good pharmacological effects, such as anti-oxidation and anti-tumor effects. ASP is one of the vital active ingredients for these pharmacological effects. Recently, studies have explored the antioxidant effect of ASPs on human OA chondrocytes induced by H_2O_2. It was found that compared with normal human chondrocytes, OA chondrocytes produce more ROS, NO, and MDA, while ASP increases the activity of iNOS, SOD, and CAT [11]. Studies have also shown that ASP can protect H9c2 cells from apoptosis induced by H_2O_2. The results are because ASP inhibits the production of ROS and lactic dehydrogenase release by activating the ATF6 pathway, thereby improving oxidative stress and endoplasmic reticulum stress levels [12]. Tian S et al. studied the antioxidant activity of ASPs obtained by the best extraction process. Taking ascorbic acid as the positive control group, they studied the effect of ASPs at different concentrations on the scavenging of several common free radicals. The results showed that the scavenging ability of hydroxyl free radicals was closely related to the concentration. When the maximum measured concentration was 800 mg/mL, the clearance rate was about 60%, close to ascorbic acid [12].

Gastrodia elata has many active components, such as gastrodin and luteolin. Since Liu reported another active component, *Gastrodia elata* polysaccharides (GP), in 1981 [13], more and more studies on GP have been reported, such as the modification of the structure [14], the neuroprotective effect [15], regulation of intestinal flora [16], and immune enhancement [17]. These research results showed that GP has excellent pharmacological effects. Recently, the anti-aging activity of GP has gradually attracted people's attention. One study extracted GP by the hot water extraction method, and chemical characterization by UV spectrometer and HPLC, then detected its antioxidant activity in vitro and in vivo. The results showed that GP could effectively remove hydroxyl radicals and has a substantial reduction ability [18]. Oral GP can increase the activities of SOD and GSH-Px in aging mice induced by D-galactose. Based on the above results, it can be concluded GP has high antioxidant activity and is beneficial in alleviating human aging.

Purslane polysaccharides (PP) can promote the production of serum cytokines and enhance the immune response in gastric cancer rats induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). PP plays an antioxidant role by increasing the activities of SOD, CAT, and GSH-Px. In addition, the protective effect on oxidative damage is dose-dependent [19]. Other researchers specially studied the free radical scavenging characteristics of PP by spectrophotometry. It was found that PP composed of glucose and galactose could scavenge superoxide anion free radicals, hydroxyl free radicals, DPPH free radicals, and NOs [20]. Free radicals have strong oxidizability, so they have a strong electron absorption ability, which will snatch the electrons of adjacent molecules and cause oxidative damage. Therefore, the free radical scavenging ability of PP has a certain application value in the antioxidation effect of diseases.

Polygonatum sibiricum polysaccharides (PSP) are considered to have an indispensable role in the treatment of diseases such as fatigue and diabetes [21]. Recent literature reported that PSP could also affect depressive behavior [22]. It was found that taking PSP can reduce the decrease of hippocampal 5-HT levels and hippocampal oxidative stress induced by lipopolysaccharides. Comprehensive analysis shows that PSP may treat depression by inhibiting ROS/HPA axis hyperfunction and inflammatory response, which can be attributed to the antioxidant effect of PSP. Some researchers specially studied the antioxidant effect of PSP [23]. In vitro tests show that the components isolated from PSP had the highest hydroxyl radical scavenging rate (78.585%) at 5 mg/mL concentration and had a strong ability to scavenge ABTS and DPPH radicals with scavenging rates of 92.794% and 75.648%, respectively. In addition, the power of chelating Fe^{2+} was up to 98.721%, which is in sharp contrast with other components. *Polygonatum odoratum*, similar to *Polygonatum sibiricum*, is also a *Liliaceae* plant with an antioxidant capacity [24]. For example, studies have explored the effect of polysaccharides isolated from *Polygonatum odoratum* (PPO) on oxidative stress induced by fatigue exercise. They have detected the changes in various biochemical parameters in serum and liver. PPO stimulates a protective effect on oxidation induced by fatigue exercise by reducing lipid peroxidation, which is attributed to the antioxidant effect of PPO.

1.2. Algal Polysaccharides

Algae are a class of substances with excellent nutrients in the ocean and are a crucial edible and medicinal resource. Polysaccharides are the main component of algae. Many biological activities of algae are closely related to algal polysaccharides. The monosaccharide composition, proportion, and molecular weight of algal polysaccharides are closely related to their biological activities, especially the sulfuric acid groups.

Five kinds of algal polysaccharides, *Ulva pertusa*, *Laminaria japonica*, *Enteromorpha linza*, *Bryopsis plumose*, and *Porphyra haitanensis* have been extracted and tested by in vitro antioxidant activity [25]. The results showed that all samples had antioxidant activity and strong free radical scavenging ability, and their scavenging effect could remain stable at high temperatures. In the test results of reduction ability, *Laminaria japonica* polysaccharides (LJP) showed the most potent reduction ability, which may be because LJP contains more sulfate and less hydroxyl, so fewer hydrogen atoms can be provided. The time-saving and straightforward microwave-assisted extraction technology is a commonly used extraction method for algae polysaccharides. The composition, properties, and biological activities of polysaccharides prepared by different extraction conditions are different and the optimal extraction conditions can be determined by response surface methodology. Fucoidan extracted from brown algae is a type of sulfated polysaccharide. The best extraction method is to extract for 15–17 min at a temperature of 120 °C and then obtain fucoidan with a concentration of 16%. The DPPH scavenging and reducing power measurement results showed that the fucoidan extracted at 90 °C has the highest antioxidant activity [26]. Similarly, *Ulva pertusa* polysaccharides were also extracted by microwave-assisted extraction technology [27]. The results of DPPH and ABTS showed that they had a strong antioxidant capacity and could improve H_2O_2− induced oxidative stress by increasing the activities of SOD and CAT.

Compared with green algae and red algae, brown algae have better antioxidant activity. Some studies have extracted fucoidan, laminaran, and alginate polysaccharides from Malaysian brown seaweeds and compared their antioxidant activities [28]. Among them, *laminaran* polysaccharides from *Sargassum polycystum* have the highest superoxide anion scavenging ability, and alginate has high DPPH scavenging activity. It shows that *Sargassum polycystum* polysaccharides may be a promising natural antioxidant. Alginate was extracted and isolated from the *Sargassum polycystum*. Lipid peroxidation levels and antioxidant enzyme activities were measured in arthritis rats to analyze whether alginate alleviates oxidative stress. The treatment of alginate can reduce the activity of various enzymes, and its antioxidant effect helps reduce inflammation in arthritis rats [29]. In addition, before the extraction of fucoidan and alginate, different fungal fermentation treatments enhance their antioxidant capacity [30]. After treatment, the molecular weight of the two polysaccharides decreased by nearly half. The content of fucose and sulfate in fucoidan and the ratio of mannuronic acid to guluronic acid (M/G) in alginate increased, suggesting that molecular weight and monosaccharide components may be the key factors affecting antioxidant capacity. Radiation is often used to degrade sodium alginate. Some researchers analyzed the effects of the ratio of M/G and the molecular weight of mannuronic acid and guluronic acid on the antioxidation ability. The results showed that the lower the molecular weight, the higher the 50% inhibition concentration [31]. Under the same molecular weight, the fraction with higher M/G has more potent antioxidant activity. It further shows that the molecular weight, content, and proportion of monosaccharide components are essential factors affecting the antioxidant properties of polysaccharides. In addition to radiation degradation, heat treatment depolymerization also obtained low molecular weight sodium alginate. ABTS and superoxide radical scavenging tests obtained similar results [32]. This suggests that low molecular weight sodium alginate is a rich and potential source of natural antioxidants.

1.3. Microbial Polysaccharides

1.3.1. Fungal Polysaccharides

Fungal polysaccharides are natural polymer compounds isolated from the fruit body, or mycelium, of fungi. They are mainly derived from medicinal mushrooms. Their excellent antioxidant effect and safe properties enable them to be widely used in medicine, food, cosmetics, and other industries.

Mushroom polysaccharides are a large class of fungal polysaccharides, mainly including *Lentinus edodes* polysaccharides (LEP), *Flammulina velutipes* polysaccharides (FVP), *Pleurotus ostreatus* polysaccharides (POP) and so on. Studies have extracted LEP with hot water extraction [33]. The results of scavenging hydroxyl free radicals, superoxide free radicals, and Fe^{2+} chelating capacity have verified that LEP has a dose-dependent antioxidant activity. There are also many reports on the effects of different molecular weights on the antioxidant activity of purified LEP [34]. By detecting the contents of various peroxidases and MDA in the liver, it was concluded that LEP has a protective effect on oxidative stress induced by D-galactose, and the effect of medium molecular weight is the best. To accurately assess the antioxidant capacity of LEP, some studies have adopted two methods of protein removal [35]. The results found that the deproteinized polysaccharides showed reduced antioxidant activity. The reduced antioxidant activity may be due to the hydrophobic cavity and fissure formed by the covalent bond between polysaccharides and protein. In addition, the study also showed that the neutral protease protein removal method was better for retaining the antioxidant activity of polysaccharides. The results of the antioxidant evaluation of polysaccharides by different extraction methods are very different. Some studies have compared the differences in antioxidant activity caused by different extraction methods of FVP [36]. It was found that enzymatic extraction has a strong hydroxyl radical scavenging and metal chelating ability, while ultrasonic extraction has a good effect on DPPH scavenging. The hot water extraction method has stronger antioxidant activity in terms of reduction ability. In another study, microwave-assisted extraction was used [37]. The effect of FVP on scavenging a variety of free radicals in vitro was analyzed. The results showed that the acid polysaccharides rich in glucose and the low average molecular weight components had stronger antioxidant potential. A study compared the relationship between the structural characteristics and antioxidant activity of polysaccharides extracted from *Pleurotus eryngii*, *Flammulina velutipes*, *Pleurotus ostreatus*, and white *Hypsizygus marmoreus* [38]. It was found that POPs rich in acid groups and with low molecular weight had the best antioxidant effect. Similarly, another study on extracting and isolating four polysaccharides from *Agaricus bisporus*, *Auricularia auricula*, *Flammulina velutipes*, and *Lentinus edodes* also showed that the polysaccharides of *Agaricus bisporus* with a low molecular weight had the most potent antioxidant activity [39].

In addition to the common *mushroom* polysaccharides, *Grifola frondosa*, a rare medicinal and edible fungus, has also attracted extensive attention. Some studies have extracted the *Grifola frondosa* polysaccharide (GFP) and separated three main polysaccharide components through chromatographic purification [40]. The results of in vitro antioxidant experiments showed that it has noticeable scavenging effects on various free radicals. In addition, strong reducing power and Fe^{2+} chelating ability were also observed. Based on these studies, some researchers have explored the effect of GFP on improving memory and anti-aging [41]. The behavioral experimental results suggested that GFP can significantly improve the memory ability of elderly rats. The pathological analysis also showed that the changes in brain histology and ultrastructure after aging are weaker, and the total antioxidant capacity and the activities of various antioxidant enzymes are improved. At the same time, it also reduced the levels of NO and MDA. The above results showed that GFP had an antioxidant ability and could effectively improve memory.

Ganoderma lucidum is widely popular because it contains a variety of highly bioactive compounds [42]. *Ganoderma lucidum* polysaccharides (GLP) with antioxidant effects have been extensively studied. Additionally, similar to the optimum drying method of other polysaccharides, the vacuum freeze-drying method used on GLP has a strong reducing power and free radical scavenging rate [43]. In many studies on the antioxidant effect of GLP, extraction methods occupy many of them. The early studies detected the antioxidant activity of ultrasonic-extracted GLP [44]. It was found that the four components separated by ultrafiltration membrane were rich in glucose and galactose. Recent studies have found that the antioxidant activity of GLP extracted by hot water extraction is stronger than that extracted by ultrasound [45]. The polysaccharides obtained by the two extraction methods have the same kind of monosaccharide, but the proportion of glucose in the polysaccharide extracted by hot water is about twice that extracted by ultrasound. There are also studies on extracting GLP by superfine grinding technology [46]. Among the three crude polysaccharides, the proportion of glucose is very similar. GLP 80 with the highest proportion of galactose has the highest antioxidant activity. Based on the above research results, this may be the result of the joint influence of the content and proportion of glucose and galactose.

In addition to the more researched fungi reviewed above, there are also other polysaccharides from *Hericium erinaceus* [47], *Cordyceps sinensis*, and *Ganoderma atrum* [48][49] that have good antioxidant activities. Activating antioxidant enzyme activity prevents mitochondrial-dependent apoptosis and plays a regulatory and protective role in colonic immune disorder and hepatotoxicity.

1.3.2. Bacterial Polysaccharides

In addition to fungal polysaccharides, bacterial polysaccharides are also a part of microbial polysaccharides. Because they are more suitable for producing extracellular polysaccharides than plants, animals, and seaweed, they have gradually attracted people’s attention [50]. The extracellular polysaccharides extracted from *Lactobacillus plantarum* have no cytotoxicity. The clearance of DPPH and lipid peroxidation can reach 64% and 66%, respectively [51]. The extracellular polysaccharides of *Brevibacterium otitidis* BTS 44 with good antioxidant activity were determined by in vitro antioxidant test [52]. The components were mannose, arabinose, glucose, and mannouronic acid, and the proportion decreased successively. An extracellular polysaccharide was extracted and purified from *Bacillus* sp. strain LBP32, which can alleviate LPS-induced inflammation by inhibiting oxidative stress [53]. *Streptomyces* isolated from soil can produce an extracellular polysaccharide mainly composed of mannose and glucose [54]. It can effectively scavenge hydroxyl radicals and DPPH radicals and has a high Fe^{2+} chelation ability. Various results showed that the extracellular polysaccharides produced by *Streptomyces* had good antioxidant activity. Some researchers studied the antioxidant activity of extracellular polysaccharides of *Streptomyces violaceus* MM72 from marine actinomycetes and obtained similar results [55]. The above results show that bacterial polysaccharides may be a good source of natural antioxidants.

1.4. Animal Polysaccharides

Animal polysaccharides are mainly derived from marine animals. Because of the unique living environment of marine animals, marine animal polysaccharides have unique structures and characteristics [56]. For example, the contents of sulfated polysaccharides in marine animal polysaccharides are higher than that in terrestrial animal polysaccharides. Many studies have proven that sulfated polysaccharides contribute to antioxidant activity [57]. In addition, compared with other marine organisms, marine animal polysaccharides usually exist in the form of covalent bonds and protein complexes. This particular structure also contributes to the antioxidant function [58].

Misgurnus anguillicaudatus polysaccharide (MAP) is a natural neutral polysaccharide in loach mucus. Early studies have proved that MAPs can scavenge various ROS, and their antioxidant activity can significantly reduce damage to the DNA chain by hydroxyl radicals. Based on this, some researchers have studied the therapeutic effects of MAP on diabetes and found that MAP can significantly scavenge superoxide anion free radicals and hydroxyl free radicals in a dose-dependent manner [59]. The results showed that its antioxidant and anti-glycosylation capabilities could protect the body from diabetes-induced oxidative damage.

Two kinds of chondroitin sulfate were extracted and isolated from two kinds of sea cucumber [60]. They can scavenge free radicals in a dose-dependent manner, which is suitable proof of the antioxidant capacity of animal polysaccharides.

Animal polysaccharides exist in mucus, cartilage, and the skin, such as *Rana chensinensis* skin. Some researchers have optimized the best process for extracting polysaccharides from *Rana chensinensis* skin (RCSP) [61]. Under the conditions of extraction temperature at 100 °C, water–material ratio of 60:1, extraction frequency of 1, and extraction time of 4.96 h, a yield of RCSP of about 2.03% can be obtained. In vitro experiments showed that RCSP had significant scavenging effects on superoxide anion and DPPH and showed a strong reducing ability. Similar results were obtained in another study [62]. The preliminary characterization experiment showed that RCSP was composed of glucose, galactose, and mannose (87.82:2.77:1.54), and its molecular weight was 12.8 kDa. The antioxidant experiment in vitro showed good free radical scavenging activity. The antioxidant experiment in vivo showed that it increased the antioxidant enzyme activity in the liver and serum of mice induced by D-galactose and decreased the level of MDA. Therefore, RCSP is a potent natural antioxidant that can be used in food and medicine.

Chitosan is a natural polysaccharide with multiple biological activities. It can be produced from shellfish, shrimps, and crabs and can also be produced from microorganisms [63]. Chitosan is an excellent N-center free radical scavenger, which can scavenge peroxide free radicals and has a good antioxidant protection effect on human serum albumin [64]. Low molecular weight chitosan can inhibit neutrophil activation and play a significant role in serum albumin oxidation, reducing the oxidative stress associated with uremia. The sources and main monosaccharides of representative polysaccharides with antioxidant activity are shown in **Figure 1**.


Lycium barbarum polysaccharide
D-Galactose L-Arabinose D-Galacturonic acid
Astragalus polysaccharide
L-Arabinose D-Galactose D-Glucose
Dendrobium officinale polysaccharide
D-Mannose D-Glucose
Angelica sinensis polysaccharide
D-Glucose L-Arabinose D-Galacturonic acid
Gastrodia elata polysaccharide
D-Glucose
Salvia miltiorrhiza polysaccharide
D-Glucose D-Galactose D-Mannose
Momordica charantia polysaccharide
D-Glucose D-Galacturonic acid D-Galactose
Sargassumhorneri polysaccharide
D-Fucose D-Mannose D-Galactose
Enteromorpha prolifera polysaccharide
D-Xylose D-Rhamnose D-Glycuronate
Ulva pertusa polysaccharide
D-Rhamnose D-Glucose D-Galactose
Lentinus edodes polysaccharide
D-Xylose D-Galactose D-Glucose
Flammulina velutipes polysaccharide
D-Galactose D-Glucose D-Mannose
Grifola frondose polysaccharide
D-Galactose D-Glucose D-Mannose
Ganoderma lucidum polysaccharide
D-Galactose D-Glucose D-Mannose
Apostichopus japonicus polysaccharide
D-Glucose D-Glycuronate D-Mannose
Rana chensinensis polysaccharide
D-Galactose D-Glucose D-Mannose

Figure 1. Sources and main monosaccharides of representative natural polysaccharides.

2. Antioxidant Activity of Natural Polysaccharide Derivatives

2.1. Sulfated Polysaccharides

Sulfated polysaccharides are important derivatives of polysaccharides. The most common methods are the chlorosulfonic acid-pyridine method (CSA-Pyr) [65], phenol-sulfuric acid method [66], sulfur trioxide pyridine method, and amino sulfonic acid-pyridine method (ASA-Pyr) [67][68]. The sulfation modification methods of neutral polysaccharides and acidic polysaccharides are different. Neutral polysaccharides can be dissolved in organic solvents and then sulfate esterification is used to obtain sulfated polysaccharides. Acidic polysaccharides have fewer hydroxyl groups and are difficult to dissolve in organic solvents, so they need to be reorganized with acidic resins and then sulfated to obtain sulfated polysaccharides with a high degree of substitution. The neutral polysaccharides and acidic polysaccharides of *Auricularia auricula* polysaccharides (AAP) were sulfated by sulfation [69]. The completion of sulfation was successfully proved by ion chromatography and infrared spectroscopy. The results of antioxidant experiments in vitro showed that both had stronger antioxidant activity than the natural AAP without sulfation modification, especially in scavenging hydroxyl free radicals and lipid peroxidation. Studies have been performed on the sulfation modification of *Flammulina velutipes* polysaccharides (FVP) [70]. The results of structural characterization and antioxidant experiments in vivo and in vitro showed that the characteristic functional groups and monosaccharide composition of FVP have changed. Studies also found sulfated FVP has more antioxidant effects than natural polysaccharides and could improve aging and inflammatory responses. Some researchers are also interested in the sulfation modification of plant polysaccharides [71]. Sulfated *Momordica charantia* polysaccharides with different substitution positions were synthesized by CSA-Pyr. It was found that C-6 substitution had more advantages than C-2 substitution through spectral analysis. In addition, both of them have more antioxidant activity than natural *Momordica charantia* polysaccharides. It is concluded that a high degree of substitution and medium molecular weight significantly affects improving antioxidant activity. The effects of sulfated polysaccharides on antioxidant activity from different angles are not all positive. For example, sulfated *pumpkin* polysaccharides with different degrees of substitution are only superior to *pumpkin* polysaccharides in scavenging superoxide anions. Their reducing power is lower than *pumpkin* polysaccharides, even with no scavenging ability to hydroxyl radicals [72]. There are studies on the sulfation modification of *Cyclocarya paliurus* polysaccharides. In a study, the derivatives with the best antioxidant properties have the lowest degree of substitution and the highest relative molecular mass and protein content [73]. The degree of substitution, relative molecular weight, sulfate content, and protein of modified polysaccharides combined may influence the degree of antioxidant. Among animal polysaccharides, the in vitro experiments of sulfated chitosan with different modified sites also showed more potent antioxidant activity than chitosan. The inhibition of deoxyribose oxidation was dose-dependent, which could effectively alleviate oxidative stress, but safety needs to be evaluated in vivo [74].

2.2. Phosphorylated Polysaccharides

Several commonly used phosphorylation reagents include phosphorus oxychloride, phosphate, phosphoric acid and anhydride, and phosphorus pentoxide. Generally, the characteristic peaks obtained by Fourier-transform infrared spectroscopy and nuclear magnetic resonance are used for qualitative analysis of polysaccharides [75]. Scientists have reported the phosphorylation modification of a variety of polysaccharides. For example, *pumpkin* polysaccharides were modified with phosphorus oxychloride-pyridine to obtain phosphorylated pumpkin polysaccharides [76]. Compared with unmodified *pumpkin* polysaccharides, phosphate-modified *pumpkin* polysaccharides have higher superoxide and hydroxyl radicals scavenging ability. Some studies came to similar conclusions about ginseng polysaccharides using the same method [77]. Studies on the phosphorylation of garlic polysaccharides indicated that the higher concentration of phosphorylated garlic polysaccharides, the stronger the antioxidant capacity within a certain range [78]. In the animal tissue oxidative damage protection experiment of phosphorylated *Momordica charantia* polysaccharides, the content of antioxidant enzymes in mouse serum, liver, and other tissues had increased [79]. These results indirectly proved that sulfated *Momordica charantia* polysaccharides had a strong antioxidant ability. This feature has also been proven in the *Pleurotus ostreatus* of fungal polysaccharides, which can effectively treat chemical liver toxicity by improving the antioxidant enzyme activity and reducing the content of MDA in the liver [80]. These provide strong evidence for the enhanced antioxidant activity of phosphorylated polysaccharides.

2.3. Carboxymethylated Polysaccharides

Carboxymethylation is a common modification method of polysaccharides to improve water solubility and biological activity. The aqueous medium and organic solvent methods are the most common synthesis methods of carboxymethylation. The aqueous medium method dissolves the polysaccharides with an alkaline solution, adds monochloroacetic acid (MCA) to mix and react for several hours, and adds an acid solution to adjust the pH to a neutral range. Ethanol is the precipitation solvent, and carboxymethyl polysaccharide derivatives can be obtained after dialysis and freeze-drying [81]. This method is unstable and takes a long time, but it is cheap and commonly used. The organic solvent method is to dissolve polysaccharides in organic solvents such as isopropanol or ethanol, and then add MCA for etherification reaction to obtain carboxymethyl polysaccharide derivatives [82]. The reaction is stable and rapid, but the price is relatively expensive with the toxic organic reagent. Carboxymethyl-modified *pumpkin* polysaccharides prepared by the aqueous medium method were proven to have a good scavenging ability of superoxide anion free radicals and hydroxyl free radicals (Liu & Huang, 2019). It has been reported that the carboxymethylated polysaccharides with a molecular weight of 354 kDa were obtained by the chemical modification of *Sargassum fusiforme* polysaccharides [83]. Compared with the unmodified *Sargassum fusiforme* polysaccharides, the modified carboxymethyl polysaccharides have a stronger free radical scavenging ability and total antioxidant activity. Similarly, the crude polysaccharides of *Enteromorpha prolifera* were degraded with hydrogen peroxide or ascorbic acid and modified by carboxymethyl [84]. The reaction conditions of carboxymethylation were optimized by the response surface method. The product was characterized and analyzed, and carboxymethylated *Enteromorpha prolifera* polysaccharides with a degree of substitution of 0.849 were obtained. The carboxymethylated *Enteromorpha prolifera* polysaccharides had stronger antioxidant activity evaluated by DPPH, hydroxyl radical, and superoxide anion radical scavenging ability. Similar conclusions have also been proven in the studies of various carboxymethylated polysaccharides [85].

2.4. Other Modification Methods

In addition, studies on multiple derivatives of the same polysaccharides also showed that the modified polysaccharide derivatives had better antioxidant activity than those without modification [86][87]. In addition to the above modification methods, acetylation modification, hydroxy-propylation modification, selenization modification, sulfonation modification, ammonium modification, and other modification methods can also affect the water solubility and biological activity of polysaccharides [79]. The commonly used chemical modification methods of natural polysaccharides are shown in **Figure 2**.

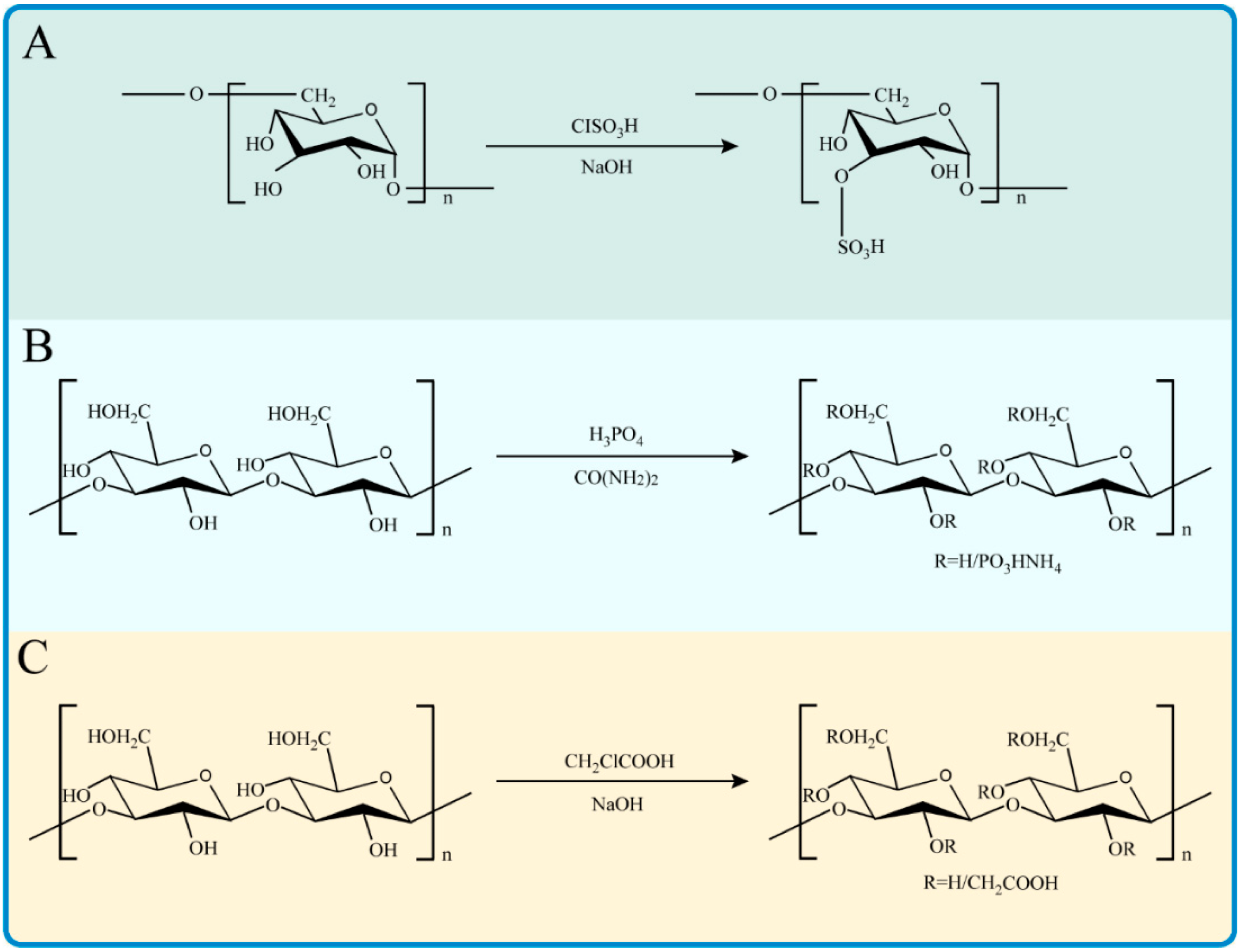

Figure 2. Several common modification methods and schematic diagrams of natural polysaccharides. (**A**) Sulfation modification with CSA; (**B**) Phosphorylation modification with phosphoric acid; (**C**) Carboxymethylation modification with monochloroacetic acid.

References

1. Tian, X.; Liang, T.; Liu, Y.; Ding, G.; Zhang, F.; Ma, Z.; Extraction, Structural Characterization, and Biological Functions of Lycium Barbarum Polysaccharides: A Review. *Biomolecules.* **2019**, *9*, 389.
2. Liu, J.; Pu, Q.; Qiu, H.; Di, D.; Polysaccharides isolated from Lycium barbarum L. by integrated tandem hybrid membrane technology exert antioxidant activities in mitochondria. *Ind. Crops Prod.* **2021**, *168*, 113547.
3. Li, J.; Shi, M.; Ma, B.; Zheng, Y.; Niu, R.; Li, K.; Protective effects of fraction 4a of polysaccharides isolated from Lycium barbarum against KBrO3-induced renal damage in rats. *Food Funct.* **2017**, *8*, 2566–2572.
4. Yan, H.; Xie, Y.; Sun, S.; Sun, X.; Ren, F.; Shi, Q.; Wang, S.; Zhang, W.; Li, X.; Zhang, J.; et al. Chemical analysis of Astragalus mongholicus polysaccharides and antioxidant activity of the polysaccharides. *Carbohydr. Polym.* **2021**, *82*, 636–640.
5. Shang, H.; Chen, S.; Li, R.; Zhou, H.; Wu, H.; Song, H.; Influences of extraction methods on physicochemical characteristics and activities of Astragalus cicer L. polysaccharides. *Process Biochem.* **2018**, *73*, 220–227.
6. Awad, A.; Khalil, S.R.; Hendam, B.M.; Abd El-Aziz, R.M.; Metwally, M.M.M.; Imam, T.S.; Protective potency of Astragalus polysaccharides against tilmicosin- induced cardiac injury via targeting oxidative stress and cell apoptosis-encoding pathways in rat. *Env. Sci. Pollut. Res. Int.* **2020**, *27*, 20861–20875.
7. Zhang, Y.; Zhang, L.; Liu, J.; Liang, J.; Si, J.; Wu, S.; Dendrobium officinale leaves as a new antioxidant source. *J. Funct. Foods.* **2017**, *37*, 400–415.
8. Fan, H.; Meng, Q.; Xiao, T.; Zhang, L.; Partial characterization and antioxidant activities of polysaccharides sequentially extracted from Dendrobium officinale. *J. Food Meas. Charact.* **2018**, *12*, 1054–1064.
9. Meng, Q.; Fan, H.; Li, Y.; Zhang, L.; Effect of drying methods on physico-chemical properties and antioxidant activity of Dendrobium officinale. *J. Food Meas. Charact.* **2017**, *12*, 1-10.
10. Huang, K.; Li, Y.; Tao, S.; Wei, G.; Huang, Y.; Chen, D.; Wu, C.; Purification, Characterization and Biological Activity of Polysaccharides from Dendrobium officinale. *Molecules.* **2016**, *21*, 701.
11. Zhuang, C.; Wang, Y.; Zhang, Y.; Xu, N.; Oxidative stress in osteoarthritis and antioxidant effect of polysaccharide from angelica sinensis. *Int. J. Biol. Macromol.* **2018**, *115*, 281-286.
12. Tian, S.; Hao, C.; Xu, G.; Yang, J.; Sun, R.; Optimization conditions for extracting polysaccharide from Angelica sinensis and its antioxidant activities. *J. Food Drug Anal.* **2017**, *25*, 766–775.
13. Liu, C.Y.; The changes of the polysaccharide and its distribution in the development process of Gastrodia elate. *Acta Bot.* **1981**, *3*, 375–380.

14. Zhu, Z.Y.; Chen, C.J.; Sun, H.Q.; Chen, L.J.; Structural characterisation and ACE-inhibitory activities of polysaccharide from Gastrodia elata Blume. *Nat. Prod. Res.* **2019**, *33*, 1721–1726.

15. Zhou, B.; Tan, J.; Zhang, C.; Wu, Y.; Neuroprotective effect of polysaccharides from Gastrodia elata blume against corticosteroneinduced apoptosis in PC12 cells via inhibition of the endoplasmic reticulum stressmediated pathway. *Mol. Med. Rep.* **2018**, *17*, 1182–1190.

16. Huo, J.; Lei, M.; Li, F.; Hou, J.; Zhang, Z.; Long, H.; Zhong, X.; Liu, Y.; Xie, C.; Wu, W.; et al. Structural Characterization of a Polysaccharide from Gastrodia elata and Its Bioactivity on Gut Microbiota. *Molecules.* **2021**, *26*, 4443.

17. Bao, Q.; Qian, L.; Gong, C.; Shen, X.; Immune-Enhancing Activity of Polysaccharides from Gastrodia elata. *J. Food Processing Preserv.* **2017**, *41*, e13016.

18. Chen, L.; Zhang, Y.P.; Jin, L.X.; Preparation, characterization and anti-ageing activity of Gastrodia elata blume polysaccharide. *Acta Aliment.* **2018**, *47*, 210-219.

19. Li, Y.; Hu, Y.; Shi, S.; Jiang, L.; Evaluation of antioxidant and immuno-enhancing activities of Purslane polysaccharides in gastric cancer rats. *Int. J. Biol. Macromol.* **2014**, *68*, 113–116.

20. YouGuo, C.; ZongJi, S.; XiaoPing, C.; Evaluation of free radicals scavenging and immunity-modulatory activities of Purslane polysaccharides. *Int. J. Biol. Macromol.* **2009**, *45*, 448–452.

21. Cui, X.; Wang, S.; Cao, H.; Guo, H.; Li, Y.; Xu, F.; Zheng, M.; Xi, X.; Han, C.; A Review: The Bioactivities and Pharmacological Applications of Polygonatum sibiricum polysaccharides. *Molecules.* **2018**, *23*, 1170.

22. Shen, F.; Song, Z.; Xie, P.; Li, L.; Wang, B.; Peng, D.; Zhu, G.; Polygonatum sibiricum polysaccharide prevents depression-like behaviors by reducing oxidative stress, inflammation, and cellular and synaptic damage. *J. Ethnopharmacol.* **2021**, *275*, 114164.

23. Li, L.; Thakur, K.; Liao, B.Y.; Zhang, J.G.; Wei, Z.J.; Antioxidant and antimicrobial potential of polysaccharides sequentially extracted from Polygonatum cyrtonema Hua. *Int. J. Biol. Macromol.* **2018**, *114*, 317–323.

24. Yang, H.; Zhang, X.; Polysaccharides from Polygonatum odoratum strengthen antioxidant defense system and attenuate lipid peroxidation against exhaustive exercise-induced oxidative stress in mice. *Trop. J. Pharm. Res.* **2017**, *16*, 795.

25. Zhang, Z.; Wang, F.; Wang, X.; Liu, X.; Hou, Y.; Zhang, Q.; Extraction of the polysaccharides from five algae and their potential antioxidant activity in vitro. *Carbohydr. Polym.* **2010**, *82*, 118–121.

26. Yuan, Y.; Macquarrie, D.; Microwave assisted extraction of sulfated polysaccharides (fucoidan) from Ascophyllum nodosum and its antioxidant activity. *Carbohydr Polym.* **2015**, *129*, 101–107.

27. Le, B.; Golokhvast, K.S.; Yang, S.H.; Sun, S.; Optimization of Microwave-Assisted Extraction of Polysaccharides from Ulva pertusa and Evaluation of Their Antioxidant Activity. *Antioxidants.*

2019, *8*, 129.

28. Mohd Fauziee, N.A.; Chang, L.S.; Wan Mustapha, W.A.; Md Nor, A.R.; Lim, S.J.; Functional polysaccharides of fucoidan, laminaran and alginate from Malaysian brown seaweeds (Sargassum polycystum, Turbinaria ornata and Padina boryana). *Int. J. Biol. Macromol.* **2021**, *167*, 1135–1145.
29. Sarithakumari, C.H.; Renju, G.L.; Kurup, G.M.; Anti-inflammatory and antioxidant potential of alginic acid isolated from the marine algae, Sargassum wightii on adjuvant-induced arthritic rats. *Inflammopharmacology* **2013**, *21*, 261–268.
30. Hifney, A.F.; Fawzy, M.A.; Abdel-Gawad, K.M.; Gomaa, M.; Upgrading the antioxidant properties of fucoidan and alginate from Cystoseira trinodis by fungal fermentation or enzymatic pretreatment of the seaweed biomass. *Food Chem.* **2018**, *269*, 387–395.
31. Sen, M.; Effects of molecular weight and ratio of guluronic acid to mannuronic acid on the antioxidant properties of sodium alginate fractions prepared by radiation-induced degradation. *Appl. Radiat. Isot.* **2011**, *69*, 126–129.
32. Kelishomi, Z.H.; Goliaei, B.; Mahdavi, H.; Nikoofar, A.; Rahimi, M.; Moosavi-Movahedi, A.A.; Mamashli, F.; Bigdeli, B.; Antioxidant activity of low molecular weight alginate produced by thermal treatment. *Food Chem.* **2016**, *196*, 897–902.
33. Chen, H.; Ju, Y.; Li, J.; Yu, M.; Antioxidant activities of polysaccharides from Lentinus edodes and their significance for disease prevention. *Int. J. Biol. Macromol.* **2012**, *50*, 214–218.
34. You, R.; Wang, K.; Liu, J.; Liu, M.; Luo, L.; Zhang, Y.; A comparison study between different molecular weight polysaccharides derived from Lentinus edodes and their antioxidant activities in vivo. *Pharm Biol.* **2011**, *49*, 1298–1305.
35. Li, S.; Wang, A.; Liu, L.; Tian, G.; Xu, F.; Effect of deproteinization methods on the antioxidant activity of polysaccharides extracted from Lentinus edodes stipe. *J. Food Meas. Charact.* **2019**, *13*, 1382–1389.
36. Zhang, Z.; Lv, G.; He, W.; Shi, L.; Pan, H.; Fan, L.; Effects of extraction methods on the antioxidant activities of polysaccharides obtained from Flammulina velutipes. *Carbohydr. Polym.* **2013**, *98*, 1524–1531.
37. Liu, Y.; Zhang, B.; Ibrahim, S.A.; Gao, S.S.; Yang, H.; Huang, W.; Purification, characterization and antioxidant activity of polysaccharides from Flammulina velutipes residue. *Carbohydr. Polym.* **2016**, *145*, 71–77.
38. Yan, J.; Zhu, L.; Qu, Y.; Qu, X.; Mu, M.; Zhang, M.; Muneer, G.; Zhou, Y.; Sun, L.; Analyses of active antioxidant polysaccharides from four edible mushrooms. *Int. J. Biol. Macromol.* **2019**, *123*, 945–956.

39. He, J.Z.; Ru, Q.M.; Dong, D.D.; Sun, P.L.; Chemical characteristics and antioxidant properties of crude water soluble polysaccharides from four common edible mushrooms. *Molecules.* **2012**, *17*, 4373–4387.

40. Chen, G.T.; Ma, X.M.; Liu, S.T.; Liao, Y.L.; Zhao, G.Q.; Isolation, purification and antioxidant activities of polysaccharides from Grifola frondosa. *Carbohydr. Polym.* **2012**, *89*, 61–66.

41. Chen, Z.; Tang, Y.; Liu, A.; Jin, X.; Zhu, J.; Lu, X.; Oral administration of Grifola frondosa polysaccharides improves memory impairment in aged rats via antioxidant action. *Mol. Nutr. Food Res.* **2017**, *61*, 1700313.

42. Cor, D.; Knez, Z.; Knez Hrncic, M.; Antitumour, Antimicrobial, Antioxidant and Antiacetylcholinesterase Effect of Ganoderma Lucidum Terpenoids and Polysaccharides: A Review. *Molecules.* **2018**, *23*, 649.

43. Fan, L.; Li, J.; Deng, K.; Ai, L.; Effects of drying methods on the antioxidant activities of polysaccharides extracted from Ganoderma lucidum. *Carbohydr. Polym.* **2012**, *87*, 1849–1854.

44. Ma, C.-w.; Feng, M.; Zhai, X.; Hu, M.; You, L.; Luo, W.; Zhao, M.; Optimization for the extraction of polysaccharides from Ganoderma lucidum and their antioxidant and antiproliferative activities. *J. Taiwan Inst. Chem. Eng.* **2013**, *44*, 886–894.

45. Kang, Q.; Chen, S.; Li, S.; Wang, B.; Liu, X.; Hao, L.; Lu, J.; Comparison on characterization and antioxidant activity of polysaccharides from Ganoderma lucidum by ultrasound and conventional extraction. *Int. J. Biol. Macromol.* **2019**, *124*, 1137–1144.

46. Kan, Y.; Chen, T.; Wu, Y.; Wu, J.; Wu, J.; Antioxidant activity of polysaccharide extracted from Ganoderma lucidum using response surface methodology. *Int. J. Biol. Macromol.* **2015**, *72*, 151–157.

47. Liao, B.; Huang, H.; Structural characterization of a novel polysaccharide from Hericium erinaceus and its protective effects against H2O2-induced injury in human gastric epithelium cells. *J. Funct. Foods* **2019**, *56*, 265–275.

48. Fan, S.; Huang, X.; Wang, S.; Li, C.; Zhang, Z.; Xie, M.; Nie, S.; Combinatorial usage of fungal polysaccharides from Cordyceps sinensis and Ganoderma atrum ameliorate drug-induced liver injury in mice. *Food Chem Toxicol.* **2018**, *119*, 66–72.

49. Fan, S.T.; Nie, S.P.; Huang, X.J.; Wang, S.; Hu, J.L.; Xie, J.H.; Nie, Q.X.; Xie, M.Y.; Protective properties of combined fungal polysaccharides from Cordyceps sinensis and Ganoderma atrum on colon immune dysfunction. *Int. J. Biol. Macromol.* **2018**, *114*, 1049–1055.

50. Donot, F.; Fontana, A.; Baccou, J.C.; Schorr-Galindo, S.; Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics and extraction. *Carbohydr. Polym.* **2012**, *87*, 951–962.

51. Mahdhi, A.; Leban, N.; Chakroun, I.; Chaouch, M.A.; Hafsa, J.; Fdhila, K.; Mahdouani, K.; Majdoub, H.; Extracellular polysaccharide derived from potential probiotic strain with antioxidant and antibacterial activities as a prebiotic agent to control pathogenic bacterial biofilm formation. *Microb Pathog.* **2017**, *109*, 214–220.
52. Asker, M.M.S.; Shawky, B.T.; Structural characterization and antioxidant activity of an extracellular polysaccharide isolated from Brevibacterium otitidis BTS 44. *Food Chem.* **2010**, *123*, 315–320.
53. Diao, Y.; Xin, Y.; Zhou, Y.; Li, N.; Pan, X.; Qi, S.; Qi, Z.; Xu, Y.; Luo, L.; Wan, H.et al.; et al. Extracellular polysaccharide from Bacillus sp. strain LBP32 prevents LPS-induced inflammation in RAW 264.7 macrophages by inhibiting NF-kappaB and MAPKs activation and ROS production. *Int Immunopharmacol.* **2014**, *18*, 12-19.
54. Elnahas, M.O.; Amin, M.A.; Hussein, M.M.D.; Shanbhag, V.C.; Ali, A.E.; Wall, J.D.; Isolation, Characterization and Bioactivities of an Extracellular Polysaccharide Produced from Streptomyces sp. MOE6. *Molecules.* **2017**, *22*, 1396.
55. Manivasagan, P.; Sivasankar, P.; Venkatesan, J.; Senthilkumar, K.; Sivakumar, K.; Kim, S.K.; Production and characterization of an extracellular polysaccharide from Streptomyces violaceus MM72. *Int. J. Biol. Macromol.* **2013**, *59*, 29-38.
56. Xiong, Q.; Song, Z.; Hu, W.; Liang, J.; Jing, Y.; He, L.; Huang, S.; Wang, X.; Hou, S.; Xu, T.et al.; et al. Methods of extraction, separation, purification, structural characterization for polysaccharides from aquatic animals and their major pharmacological activities. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 48–63.
57. Xue, W.; Zeng, Q.; Lin, S.; Zan, F.; Hao, T.; Lin, Y.; van Loosdrecht, M.C.M.; Chen, G.; Recovery of high-value and scarce resources from biological wastewater treatment: Sulfated polysaccharides. *Water Res.* **2019**, *163*, 114889.
58. Pomin, V.H.; Review: An overview about the structure-function relationship of marine sulfated homopolysaccharides with regular chemical structures. *Biopolymers.* **2009**, *91*, 601–609.
59. Zhang, L.S.; Wang, X.; Dong, L.L.; Antioxidation and antiglycation of polysaccharides from Misgurnus anguillicaudatus. *Food Chem.* **2011**, *124*, 183–187.
60. Zou, S.; Pan, R.; Dong, X.; He, M.; Wang, C.; Physicochemical properties and antioxidant activities of two fucosylated chondroitin sulfate from sea cucumber Acaudina molpadioidea and Holothuria nobilis. *Process Biochem.* **2016**, *51*, 650–658.
61. Wang, Z.; Zhao, Y.; Su, T.; Extraction and antioxidant activity of polysaccharides from Rana chensinensis skin. *Carbohydr Polym.* **2015**, *115*, 25–31.
62. Wang, Z.; Zhao, Y.; Su, T.; Zhang, J.; Wang, F.; Characterization and antioxidant activity in vitro and in vivo of polysaccharide purified from Rana chensinensis skin. *Carbohydr Polym.* **2015**, *126*, 17–22.

63. Ghormade, V.; Pathan, E.K.; Deshpande, M.V.; Can fungi compete with marine sources for chitosan production?. *Int. J. Biol. Macromol.* **2017**, *104*, 1415–1421.

64. Anraku, M.; Kabashima, M.; Namura, H.; Maruyama, T.; Otagiri, M.; Gebicki, J.M.; Furutani, N.; Tomida, H.; Antioxidant protection of human serum albumin by chitosan. *Int. J. Biol. Macromol.* **2008**, *43*, 159–164.

65. Huang, R.; Shen, M.; Yu, Y.; Liu, X.; Xie, J.; Physicochemical characterization and immunomodulatory activity of sulfated Chinese yam polysaccharide. *Int. J. Biol. Macromol.* **2020**, *165*, 635–644.

66. Chen, L.; Huang, G.; Antioxidant activities of sulfated pumpkin polysaccharides. *Int. J. Biol. Macromol.* **2019**, *126*, 743–746.

67. Li, H.; Wang, X.; Xiong, Q.; Yu, Y.; Peng, L.; Sulfated modification, characterization, and potential bioactivities of polysaccharide from the fruiting bodies of Russula virescens. *Int. J. Biol. Macromol.* **2020**, *154*, 1438–1447.

68. Xu, Y.; Gao, Y.; Liu, F.; Niu, X.; Wang, L.; Li, X.; Chen, H.; Yang, Y.; Sulfated modification of the polysaccharides from blackcurrant and their antioxidant and alpha-amylase inhibitory activities. *Int. J. Biol. Macromol.* **2018**, *109*, 1344–1354.

69. Zhang, H.; Wang, Z.Y.; Yang, L.; Yang, X.; Wang, X.; Zhang, Z.; In vitro antioxidant activities of sulfated derivatives of polysaccharides extracted from Auricularia auricular. *Int. J. Mol. Sci.* **2011**, *12*, 3288–3302.

70. Yuan, F.; Gao, Z.; Liu, W.; Li, H.; Zhang, Y.; Feng, Y.; Song, X.; Wang, W.; Zhang, J.; Huang, C. et al.; et al. Characterization, Antioxidant, Anti-Aging and Organ Protective Effects of Sulfated Polysaccharides from Flammulina velutipes. *Molecules.* **2019**, *24*, 3517.

71. Liu, X.; Chen, T.; Hu, Y.; Li, K.; Yan, L.; Catalytic synthesis and antioxidant activity of sulfated polysaccharide from Momordica charantia L. *Biopolymers.* **2014**, *101*, 210–215.

72. Chen, L.; Huang, G.; Antioxidant activities of sulfated pumpkin polysaccharides. *Int. J. Biol. Macromol.* **2019**, *126*, 743–746.

73. Xie, J.H.; Wang, Z.J.; Shen, M.Y.; Nie, S.P.; Gong, B.; Li, H.S.; Zhao, Q.; Li, W.J.; Xie, M.Y.; Sulfated modification, characterization and antioxidant activities of polysaccharide from Cyclocarya paliurus. *Food Hydrocoll.* **2016**, *53*, 7–15.

74. Xing, R.; Yu, H.; Liu, S.; Zhang, W.; Zhang, Q.; Li, Z.; Li, P.; Antioxidant activity of differently regioselective chitosan sulfates in vitro. *Bioorg Med Chem.* **2005**, *13*, 1387–1392.

75. Xia, S.; Zhai, Y.; Wang, X.; Fan, Q.; Dong, X.; Chen, M.; Han, T.; Phosphorylation of polysaccharides: A review on the synthesis and bioactivities. *Int. J. Biol. Macromol.* **2021**, *184*, 946–954.

76. Chen, L.; Huang, G.; Antioxidant activities of phosphorylated pumpkin polysaccharide. *Int. J. Biol. Macromol.* **2019**, *125*, 256–261.
77. Xiong, X.; Huang, G.; Huang, H.; The antioxidant activities of phosphorylated polysaccharide from native ginseng. *Int. J. Biol. Macromol.* **2019**, *126*, 842–845.
78. Chen, J.; Huang, G.; Antioxidant activities of garlic polysaccharide and its phosphorylated derivative. *Int. J. Biol. Macromol.* **2019**, *125*, 432–435.
79. Chen, F.; Huang, G.; Huang, H.; Preparation, analysis, antioxidant activities in vivo of phosphorylated polysaccharide from Momordica charantia. *Carbohydr. Polym.* **2021**, *252*, 117179.
80. Duan, Z.; Zhang, Y.; Zhu, C.; Wu, Y.; Du, B.; Ji, H.; Structural characterization of phosphorylated Pleurotus ostreatus polysaccharide and its hepatoprotective effect on carbon tetrachloride-induced liver injury in mice. *Int. J. Biol. Macromol.* **2020**, *162*, 533–547.
81. Liu, Y.; Huang, G.; The antioxidant activities of carboxymethylated cushaw polysaccharide. *Int. J. Biol. Macromol.* **2019**, *121*, 666–670.
82. Li, S.; Xiong, Q.; Lai, X.; Li, X.; Wan, M.; Zhang, J.; Yan, Y.; Cao, M.; Lu, L.; Guan, J.et al.; et al. Molecular Modification of Polysaccharides and Resulting Bioactivities. *Compr. Rev. Food Sci. Food Saf.* **2016**, *15*, 237–250.
83. Li, Y.T.; Chen, B.J.; Wu, W.D.; Ge, K.; Wei, X.Y.; Kong, L.M.; Xie, Y.Y.; Gu, J.P.; Zhang, J.C.; Zhou, T.; et al. Antioxidant and antimicrobial evaluation of carboxymethylated and hydroxamated degraded polysaccharides from Sargassum fusiforme. *Int. J. Biol. Macromol.* **2018**, *118*, 1550–1557.
84. Shi, M.J.; Wei, X.; Xu, J.; Chen, B.J.; Zhao, D.Y.; Cui, S.; Zhou, T.; Carboxymethylated degraded polysaccharides from Enteromorpha prolifera: Preparation and in vitro antioxidant activity. *Food Chem.* **2017**, *215*, 76–83.
85. Gao, H.; Huang, G.; Preparation and antioxidant activity of carboxymethylated garlic polysaccharide. *Int. J. Biol. Macromol.* **2019**, *121*, 650–654.
86. Cao, Y.Y.; Ji, Y.H.; Liao, A.M.; Huang, J.H.; Thakur, K.; Li, X.L.; Hu, F.; Zhang, J.G.; Wei, Z.J.; Effects of sulfated, phosphorylated and carboxymethylated modifications on the antioxidant activities in-vitro of polysaccharides sequentially extracted from Amana edulis. *Int. J. Biol. Macromol.* **2020**, *146*, 887–896.
87. Chen, F.; Huang, G.; Yang, Z.; Hou, Y.; Antioxidant activity of Momordica charantia polysaccharide and its derivatives. *Int. J. Biol. Macromol.* **2019**, *138*, 673–680.