



## Review

## Chemically modified polysaccharides: Synthesis, characterization, structure activity relationships of action

Yong Xu<sup>a</sup>, Yu-ji Wu<sup>a</sup>, Pei-long Sun<sup>a</sup>, Fu-ming Zhang<sup>b</sup>, Robert J. Linhardt<sup>b,c</sup>, An-qiang Zhang<sup>a,\*</sup><sup>a</sup> Department of Food Science and Technology, Zhejiang University of Technology, Hangzhou 310014, China<sup>b</sup> Department of Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA<sup>c</sup> Departments of Chemistry and Chemical Biology and Biomedical Engineering, Biological Science, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

## ARTICLE INFO

## Article history:

Received 17 December 2018

Received in revised form 22 March 2019

Accepted 27 March 2019

Available online 06 April 2019

## Keywords:

Chemical modification

Polysaccharides

Preparation methods

Characterization

Bioactivities

Structure activity relations of action

## ABSTRACT

Polysaccharides are a major class of biomacromolecules. Their bioactivities depend on chemical structure, which includes monosaccharide composition, linkages below sugar residues, and solution conformation. Many researchers report that chemical modifications of polysaccharides lead to a significantly increase in the structural diversity, promoting bioactivity and even add new bioactivities, including antioxidant and anti-tumor properties as well as anticoagulant and immunoregulatory activities. This paper reviews the recent progress of chemical modification of polysaccharides, including i) the common synthetic methods of chemical modification; ii) their structural characterization; iii) their bioactivities; and iv) the structure activity relationships of these modified polysaccharides. This review also suggests future directions for researchers and new applications for chemically modified polysaccharide derivatives in the pharmaceutical and food industries.

© 2019 Elsevier B.V. All rights reserved.

## Contents

1.	Introduction	971
2.	Methods of chemical modification of polysaccharides	971
2.1.	Sulfated modification	971
2.1.1.	Chlorosulfonic acid-pyridine method	971
2.1.2.	Sulfuric acid method	972
2.1.3.	Sulfur trioxide-pyridine method	972
2.2.	Phosphorylation	972
2.2.1.	Phosphoric acid and its anhydride method	972
2.2.2.	Phosphorus oxychloride method	972
2.2.3.	Phosphates method	972
2.2.4.	Phosphorus pentoxide method	972
2.3.	Carboxymethylation	972
2.4.	Selenization	973
2.5.	Methylation	973
2.6.	Acetylation	973
2.7.	Other chemical modifications	973
3.	Structure characterization	973
3.1.	Basic characteristics of successful modification	973
3.2.	Structural characterization of chemically modified polysaccharides	973
4.	Structure activity relationships of chemical modified polysaccharides	974
4.1.	Antioxidant activity	974
4.1.1.	DPPH radical assay	974
4.1.2.	Hydroxyl radical assay	974

\* Corresponding author.

E-mail address: [zhanganqiang@zjut.edu.cn](mailto:zhanganqiang@zjut.edu.cn) (A. Zhang).

4.1.3.	Superoxide radical assay . . . . .	974
4.1.4.	Reducing power assay . . . . .	974
4.2.	Anticoagulant activity . . . . .	974
4.3.	Anti-tumor activity . . . . .	975
4.4.	Immunoregulatory activity . . . . .	975
4.5.	Anti-viral activity . . . . .	975
4.6.	Antibacterial activities . . . . .	975
4.7.	Others . . . . .	975
5.	Conclusions . . . . .	975
	Acknowledgements . . . . .	975
	Conflict of interest . . . . .	975
	References . . . . .	976

## 1. Introduction

Polysaccharides are natural macromolecular polymers that are biosynthesized in plants, animals and microorganisms [1]. A polysaccharide generally consists of >10 monosaccharides joined by glycosidic bonds in either unbranched (linear) or branched chains [2]. Many methods have been used for extraction and preparation of polysaccharides, including hot-water extraction, acid extraction, and alkali extraction [3]. Numerous studies have shown that polysaccharides can exhibit antioxidant [4], anti-tumor [5], anticoagulant [6], anti-virus [7], anti-radiation [8], anti-cancer [9] and immunoregulatory [10] activities. This wide variety of useful biological properties suggest the potential nutritional and pharmaceutical benefits of polysaccharides as healthy foods and therapeutics.

Many natural polysaccharides lack useful bioactivities or exhibit only weak bioactivities. Thus, the molecular modification of these polysaccharides offers an approach to enhance their bioactivities by changing their structural and conformational properties [11,12]. Recent studies have shown sulfation [13], phosphorylation [14], methylation [15], carboxymethylation [16], acetylation [17], hydroxy-propylation [18], selenylation [19] and etherification [20] of polysaccharides as usual methods for polysaccharide modification. The results showed that they can improve the biological properties of these polysaccharides, sometimes producing entirely new activities [21]. One example of a biologically active polysaccharide is chondroitin sulfate (CS), which is already approved therapeutic treatment of articular cartilage osteoarthritis [22].

There are several recent reviews that discuss the chemical modification of polysaccharides used to enhance their bioactivities [23–25]. In the current review, we provide an update on research for the chemical modification of polysaccharides, including synthetic methods, structural characterization, and structure activity relationships of action.

## 2. Methods of chemical modification of polysaccharides

Based on recent publications, the most common chemical modifications used on polysaccharides are summarized in Table 1.

**Table 1**  
Summary of the methods for chemical modification of polysaccharides.

Classification of modification	Methods	Reagents	Reference
Sulfation	Chlorosulfonic acid-pyridine method	Chlorosulfonic acid (CAS), anhydrous pyridine	[26]
	Sulfuric acid method	Sulfuric acid, butanol complex, ammonium sulfate	[10]
	Sulfur trioxide-pyridine method	<i>N, N</i> -dimethylformamide (DMF), SO <sub>3</sub> ·Py	[27]
Phosphorylation	Phosphoric acid and its anhydride method	DMSO, phosphoric acid	[28]
	Phosphorus oxychloride method	Phosphorus oxychloride, anhydrous formamide, anhydrous pyridine	[29]
	Phosphate method	Sodium tripolyphosphate, sodium trimetaphosphate	[30]
	Phosphorus pentoxide method	Phosphorus pentoxide, methanesulfonic acid	[31]
Carboxymethylation	Chloroacetic acid method	Chloroacetic acid, 20% NaOH, isopropanol	[32]
Selenization	NA-SS	HNO <sub>3</sub> , Na <sub>2</sub> SeO <sub>3</sub> , BaCl <sub>2</sub>	[33]
Methylation	DMSO- iodomethane method	DMSO, iodomethane	[15]
Acetylation	Acetic anhydride-pyridine system with formamide	Formamide, acetic anhydride, pyridine	[34]
Other chemical modifications	Hydroxypropylation	Isopropanol, propylene oxide	[18]
	<i>N, O</i> -carbonylation	TEMPO, NaBr, NaClO, NaHCO <sub>3</sub>	[35]

### 2.1. Sulfated modification

Sulfated polysaccharides are polyanions with sulfo groups formed through sulfonation of a polysaccharide chain [36]. Sulfates are usually introduced at the available hydroxyl groups present at C-1,2,3,4, and/or 6 of a polysaccharide. The structural characteristics of sulfated polysaccharides are considerably different from its precursor polysaccharide, which may lead to enhanced bioactivities [37]. There are several sulfonation methods available for polysaccharides, including the use of chlorosulfonic acid-pyridine, sulfuric acid, and sulfur trioxide-pyridine.

#### 2.1.1. Chlorosulfonic acid-pyridine method

The chlorosulfonic acid-pyridine method is commonly used in the sulfonation of polysaccharides. This sulfonation reagent was prepared by adding chlorosulfonic acid (CSA) dropwise to anhydrous pyridine at a specific ratio under agitation and with cooling. Polysaccharide powder was added to anhydrous formamide at a temperature with stirring, then sulfonation reagent was added dropwise. After the reaction, the mixture was cooled to room temperature, the solution was adjusted to pH 7 with NaOH. Then the supernatant was collected and dialyzed. The sulfated polysaccharide in the dialysate was precipitated with alcohol and the precipitate was dissolved in water and freeze-dried to obtain sulfated polysaccharide [26].

Studies have demonstrated that the proportion of reagents, reaction time and temperature can significantly influence the degree of substitution (DS) of sulfated derivatives. The reaction condition for polysaccharide sulfonation was optimized by using response surface method. Results showed that the reaction temperature was the most significant single parameter influencing the DS, this was followed by reaction duration, and the ratio of CSA/Pyridine [26]. Microwave-assisted synthesis of sulfated polysaccharide also has proved to be highly effective, which resulted in high DS [36]. Moreover, 4-dimethylaminopyridine/dimethyl cyclohexylcarbodiimide (DMAP/DCC) has been demonstrated to be a sufficiently effective homogeneous reaction catalyst in the sulfonation of *Artemisia sphaerocephala* polysaccharide (ASP). DMAP/DCC showed obvious improvement in the DS of sulfated samples [38]. The

amount of chlorosulfonic acid was important for DS value, as it provided sulfo groups in the sulfonation reaction. However, chlorosulfonic acid is a strong acid. During sulfonation, polysaccharides can be easily degraded under acidic conditions at high temperatures. Chlorosulfonic acid-pyridine method is still the most commonly used method to prepare sulfated polysaccharides, as it results in high DS values at high yields.

### 2.1.2. Sulfuric acid method

Sulfuric acid method of sulfonation is similar to the chlorosulfonic acid-pyridine method. A mixture of concentrated sulfuric acid and butanol complex were prepared and stirred in ice bath, and then ammonium sulfate was added. The polysaccharide powder was then slowly added dropwise. The reaction mixture was stirred for 3 h at 10 °C and then neutralized with sodium hydroxide solution and precipitated with ethanol [10]. A chemically sulfated polysaccharide from *Cordyceps gunnii mycelia* (SPS50) was prepared using sulfuric acid. The structural characteristics of the resulting sulfated polysaccharide was determined using infrared analysis (IR), high performance liquid chromatography (HPLC) and polyacrylamide gel electrophoresis (PAGE) analysis. Results indicated that the sulfo groups had been successfully introduced in polysaccharide (SPS50) [39].

Compared with the chlorosulfonic acid pyridine method, the concentrated sulfuric acid method has the advantages of stable reaction conditions and the low toxicity of the required chemical reagents. However, the disadvantages of this method include the degradation and carbonization of polysaccharides by sulfuric acid [11].

### 2.1.3. Sulfur trioxide-pyridine method

Sulfur trioxide-pyridine method is also an effective method for the sulfonation of polysaccharides. Polysaccharide powder was dissolved in *N,N*-dimethylformamide (DMF) by stirring at room temperature for 30 min before SO<sub>3</sub>·Py was added. After stirring for 3 h, the mixture solution was then neutralized with sodium hydroxide solution and precipitated with ethanol [27]. *Lycium barbarum* polysaccharide (LbGp1) was sulfonated with sulfur trioxide-pyridine complex in *N,N*-dimethylformamide. The sulfated polysaccharide product was analyzed showing that sulfo group substitutions were mainly located on C-5 of Ara, and to a lesser extent on C-4 or C-6 Gal [27]. A high DS (>2.1) of sulfated chitosan improved the anticoagulant activity. GC-MS analysis was achieved successfully by preparing the trimethylsilylated saccharide derivatives [40].

## 2.2. Phosphorylation

Phosphorylated polysaccharides are widely distributed in nature, in various forms and with complex structures. The types of natural phosphorylated polysaccharides are relatively limited, so researchers often modify polysaccharides to prepare novel phosphorylated polysaccharides derivatives [41]. The hydroxyl group of polysaccharides is replaced by phosphate group in a reaction called phosphorylation. Phosphorylation enhances the water-solubility and changes molecular weight and chain conformation of a polysaccharide because of the resulting charged phosphate groups [42]. Phosphoric acid and its anhydride [43], phosphorus oxychloride [44], phosphate [45], and phosphorus pentoxide [46] are commonly used for the phosphorylation of polysaccharides.

### 2.2.1. Phosphoric acid and its anhydride method

Polysaccharide powder was dissolved in DMSO containing 8 M urea, then phosphoric acid was immediately added to the solution dropwise prior to heating. After heating to 100 °C and stirring for 6 h, the mixture was cooled to room temperature and dialyzed against distilled water [28]. Phosphorylated polysaccharides have been used as flocculants for zinc and ferric oxide particles. Results indicated that anionic phosphorylated derivatives of dextran and cellulose exhibit excellent flocculation properties [47].

### 2.2.2. Phosphorus oxychloride method

The phosphorus oxychloride is a phosphoryl chloride with high reactivity that is to prepare phosphorylated polysaccharides with high DS. Phosphorus oxychloride was added dropwise in anhydrous pyridine at a specified ratio, under agitation and cooling. The polysaccharide powder was first suspended in anhydrous formamide at room temperature with stirring. Anhydrous pyridine was added and stirred for 30 min until the polysaccharide was completely dissolved. Phosphorylation reagent was then added dropwise and after the reaction was complete, the mixture was cooled to room temperature and neutralized with NaOH. The mixtures were then precipitated with ethanol [29]. The phosphorylation of the *Artemisia sphaerocephala* polysaccharide (ASP) suggested that temperature was the key reaction condition for yielding phosphate mono-esters and/or di-esters [44]. A synthesis of phosphorylated pumpkin polysaccharides demonstrated that high POCl<sub>3</sub>/pyridine ratio under constant reaction conditions could increase the degree of substitution and no remarkable degradation occurring in the phosphorylation process [29].

### 2.2.3. Phosphates method

In the recent study, scientists used sodium tripolyphosphate and sodium trimetaphosphate to prepare phosphorylated polysaccharides [30]. Sodium tripolyphosphate and sodium trimetaphosphate at a specified ratio were dissolved in water. Polysaccharide powder was then added and NaHCO<sub>3</sub> was used to adjust the pH to 9. The mixture was reacted for 5 h at 80 °C and then precipitated with ethanol [30]. Phosphorylation of psyllium seed polysaccharide (PhPPS) resulted in a phosphorylated polysaccharide having shear thinning properties. It was suggestive of the potential use of PhPPS as gelling and suspending agent. [48]. The antiviral activity of phosphorylated *Radix Cyathulae officinalis* polysaccharide (pRCPS) prepared through phosphate phosphorylation was significantly higher than the unmodified RCPS. The antiviral activities of pRCPS were related to their level of phosphate content [49].

### 2.2.4. Phosphorus pentoxide method

Phosphorus pentoxide is a commonly used phosphorylation agent. Because of the low solubility of phosphorus pentoxide, polysaccharide phosphorylation is accomplished using methanesulfonic acid as the solvent and under low temperature. The application of this method is restricted due to the strongly acid environment and the potential for serious degradation of the polysaccharide [31].

## 2.3. Carboxymethylation

Carboxymethylation of polysaccharides involves the introduction of carboxymethyl groups into the hydroxyl groups of polysaccharides. The addition of carboxylate groups can enhance the water solubility of polysaccharides and change their conformations, further improving their bioactivities [50]. Carboxymethylation reagent was prepared by adding 2.63 g chloroacetic acid to 5 mL of 20% NaOH and 12.5 mL isopropanol. The polysaccharide powder was then suspended in 8 mL isopropanol with stirring and for 30 min at room temperature and 5 mL of 20% NaOH was added and stirred for 3 h at room temperature. The carboxymethylation reagent was finally added with stirring and reacted at 60 °C for 4 h before cooling to room temperature and precipitating the product with ethanol [32].

The carboxymethylated degraded polysaccharide from *Enteromorpha prolifera* (CDPE) was obtained by carboxymethylated modification. The reaction conditions were optimized using response surface method. Results showed that carboxymethylation enhanced the antioxidant activity of polysaccharide [51]. The DS was affected most by the concentration of monochloroacetic acid. The study found that water solubility and bioactivities of the carboxymethylated polysaccharide from *Tremella fuciformis* (CATP) were improved with the increased DS [52].

## 2.4. Selenization

Selenium is a necessary microelement for various organisms. It has biological functions as an antioxidant, a hypoglycemic, a hypolipidemic and has anti-tumor activity. Natural selenium-containing polysaccharides are found at low concentrations in plants or microorganisms. Selenization is a widely used method to introduce selenium to polysaccharide and enhance their biological activities. There are many methods for selenization of polysaccharides, including nitric acid-sodium selenite (NA-SS) [53–59], nitric acid-selenous acid (NA-SA) [19,60], glacial acetic acid-selenous acid (GA-SA) [61], glacial acetic acid-sodium selenite (GA-SS) [61] and selenium oxychloride (SOC) methods [61]. NA-SS method is frequently used for selenization due to its simple reaction conditions and excellent selenization efficiency. The polysaccharide powder was dissolved in  $\text{HNO}_3$  and stirred for half an hour at room temperature, followed by addition of  $\text{Na}_2\text{SeO}_3$  and  $\text{BaCl}_2$ . The mixture was placed on the water bath for 8 h at 75 °C and then neutralized by NaOH solution [33]. Lentinan was modified with nitric acid-sodium selenite method based on the orthogonal experiments [53]. An ultrasonic wave assisted selenization was used to prepare selenated polysaccharide from *Cordyceps militaris* [19] and *Artemisia sphaerocephala* [60].

## 2.5. Methylation

In methylation, a polysaccharide powder was fully dissolved in DMSO, and then NaOH pellets were added. After incubation under stirring for 2 h, iodomethane was added slowly under cooling. The reaction was kept at 4 °C for 3 h before water was added to stop the reaction. Chloroform was used to extract the methylated polysaccharide. The methylated product can be characterized by FT-IR spectrum, which should show the complete disappearance of the hydroxyl band at 3200–3700  $\text{cm}^{-1}$  [15].

The solubility of the polysaccharide in DMSO is a major limitation, hindering complete methylation. In the past, ultrasound and microwave were used to assist polysaccharides to dissolve in dimethylsulfoxide [62]. Swelling the samples by heating in DMSO overnight can improve the methylation of free hydroxyl groups. A number of reagents were tested to improve the solubility of polysaccharides in DMSO. In addition, glycerol was used to improve the solubility of polysaccharides for methylation [63]. Degradation of polysaccharides into monosaccharides is an effective method for methylation analysis. An example of a common chemical degradation method is Smith degradation reaction [63]. The appearance of insoluble material at the organic/aqueous interface during this partitioning may indicate the presence of non-methylated material and necessitate a double methylation procedure. Sulfated polysaccharides that have been methylated are not well distributed into chloroform and must be isolated by dialysis [64].

## 2.6. Acetylation

The hydroxyl groups of the polysaccharides units are converted by esterification into acetyl groups in the acetylation reaction. The acetyl groups could make polysaccharide branches stretch and change spatial arrangement of polysaccharide chains, further resulting in the exposure

of polysaccharide hydroxyl groups [25]. Thus, acetylation modification can modify the water solubility and hydrophobicity of polysaccharide [17].

*Artemisia sphaerocephala* Krasch Polysaccharide (ASKP) was acetylated by using acetic anhydride–pyridine system with formamide as the solvent [34]. In general, dry polysaccharide and acetic anhydride were mixed in formamide before adding pyridine as the catalyst. The resulting mixture was stirred at 25 °C for 12 h. The acetylated product was recovered by extensive dialysis against water and then dried [34]. Selection of a catalyst is important for acetylation of polysaccharide. Pyridine and 4-dimethylaminopyridine (4-DMAP) are common catalysts for esterification, but pyridine is toxic and 4-DMAP is relatively expensive. *N*-bromosuccinimide (NBS) was found to be a highly effective catalyst for acetylation of a variety of alcohols with acetic anhydride under mild reaction conditions [65]. Acetylation of pumpkin polysaccharide using acetic anhydride with pyridine and NBS as catalyst showed higher antioxidant activities than the unmodified polysaccharide [66].

## 2.7. Other chemical modifications

Hydroxypropylation is a low cost and relatively non-toxic modification. Hydroxypropyl *Ganoderma lucidum* polysaccharide (H-GLP) has stronger antioxidant properties than GLP [18]. *N,O*-Carbonylated chitosan derivative (NTCS) is synthesized by oxidation and substitution reaction. Results show that NTCS is soluble compared with chitosan [35]. Reversible deactivation radical polymerization (RDRP) is also used to modify natural polysaccharides to develop new hybrid materials, and is a growing research area with vast potential [67].

## 3. Structure characterization

### 3.1. Basic characteristics of successful modification

Modified polysaccharide product is usually first measured for DS of the polysaccharide to confirm the success of the modification reaction. DS is often assessed by infrared for a characteristic absorption peak of the modifying group. For example, the sulfated modification of longan polysaccharide (LP1-S), prepared by sulfuric acid method, shows two characteristic absorption bands (1223 and 640  $\text{cm}^{-1}$ ) in the FT-IR spectrum indicating that the sulfate derivate had been produced with a high (2.011) DS [10]. Basic characteristics of successful modifications are summarized in Table 2.

### 3.2. Structural characterization of chemically modified polysaccharides

Chemically modified polysaccharides are often prepared from crude polysaccharides that must be purified. A purified homopolysaccharides can be analyzed by using high performance liquid chromatography (HPLC) to determine its molecular weight (Mw) properties. Ultraviolet spectroscopy (UV), scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), monosaccharide analysis by derivatization followed by gas chromatography-mass spectrometer (GC-MS) and nuclear magnetic resonance (NMR) are common methods to be used for the determination of the structure of a chemical modified

**Table 2**  
The infrared characteristic absorption peaks of modified polysaccharides.

Classification of modification	DS	The characteristic absorption peak	Reference
Sulfated modification	2.0	The new peaks at 1223 and 640 $\text{cm}^{-1}$	[10]
Phosphorylated modification	0.5	The regions of 1252–1259 $\text{cm}^{-1}$ and 897–911 $\text{cm}^{-1}$	[44]
Carboxymethylated modification	0.4–1.2	The new signal at 1424 $\text{cm}^{-1}$	[50]
Selenized modification	641 $\mu\text{g/g}$	The new peaks at 926 and 840 $\text{cm}^{-1}$	[60]
Acetylation modification	0.1–0.6	A band around 1090 $\text{cm}^{-1}$	[68]
Hydroxypropylation	12.0% (w/w)	The new peaks at 2970 and 1379 $\text{cm}^{-1}$	[18]

polysaccharide. The structural characteristics identified by such analysis includes the monosaccharide residues, the position and chirality of glycosidic linkages, and the sequence of monosaccharide residues.

In monosaccharide composition, certain acidic condition during sulfation and phosphorylation caused hydrolysis of the glycosidic bonds or cross-linking effect. It influenced the monosaccharide composition of modified polysaccharides, and consequently contributed to enhancing the variety of polysaccharide derivatives [44].

$^{13}\text{C}$  NMR is widely used in the study of polysaccharide structure, especially chemical shift with infrequently overlapping peaks. After modification, it was found that the  $^{13}\text{C}$  NMR spectrum became more complicated because the carbon directly attached to electron-withdrawing sulfate or phosphate group would shift to a lower field position. Meanwhile, the carbon indirectly attached to the group would shift to higher field position [13,44].

The major driving force for the conformation in aqueous solution is due to the intramolecular hydrogen bonding. The introduction of modified groups could strengthen the effect of electrostatic repulsion, resulting in the relatively expanded conformation of the modified polysaccharide derivatives. It enhances the stiffness of the chains, resulting in a relatively extended flexible chain [16,29,37].

#### 4. Structure activity relationships of chemical modified polysaccharides

The chemical modification can enhance bioactivities of polysaccharides, and even produce new properties (see a summary in Table 3).

##### 4.1. Antioxidant activity

The excessive free radicals produced during cell metabolism are toxic to human body. Polysaccharides play an important role as free radical scavengers in the prevention of harmful oxidative damage in human body [26,68].

###### 4.1.1. DPPH radical assay

The scavenging activity on DPPH, a stable free radical, is a widely index and a quick method to determine the free radical-scavenging activities of antioxidants [26,69]. Studies demonstrated that introduction of sulfate or phosphate groups to polysaccharides significantly increased the DPPH radical-scavenging activities [1]. A study using sulfated polysaccharide (S-SGP) SGP from *Sphallerocarpus gracilis* indicated that the sulfation in the S-SGP molecule can activate the hydrogen atom of the anomeric carbon, leading to an increase in antioxidant activity. Low molecule weight polysaccharides were found to be benefit for the scavenging activity of DPPH radicals [26]. Thus, the antioxidant activity of large polysaccharides might be sterically hindered whereas low Mw polysaccharides might be more effective [1].

###### 4.1.2. Hydroxyl radical assay

The hydroxyl radical can cross cell membranes easily and react with many biological macromolecules including DNA, polyunsaturated fatty

acid and proteins in living cells and induce severe damage as an oxidative stress injury [26,70].

Recent studies have shown that there are two types of antioxidant mechanisms capable of preventing damage by hydroxyl radicals. One inhibits the production of hydroxyl radical, and the other one scavenges the hydroxyl radical produced [14]. In the former, the antioxidant activity may ligate to the metal ions which react with  $\text{H}_2\text{O}_2$  to get the metal complexes. Thus, the metal complexes formed cannot further react with  $\text{H}_2\text{O}_2$  to generate hydroxyl radicals [14,71]. In the latter, previous studies have shown that the effect of polysaccharides on hydroxyl radical scavenging might be due to its hydroxyl group. Sulfated or phosphorylated derivatives can provide hydrogen for binding with hydroxyl radicals and form a stable radical to achieve the scavenging effect [1]. Also, the sulfation modification experiment showed that in the molecule of high DS, in which many of the  $-\text{OH}$  groups are substituted by  $-\text{OSO}_3\text{H}$  groups, the scavenging effect can be enhanced [21].

##### 4.1.3. Superoxide radical assay

The superoxide radical is a highly toxic species. It can induce hydroxyl radical formation and lipid peroxidation, both of which are harmful to biomolecules, such as DNA, enzymes and proteins [1,14].

The sulfated polysaccharides obtained from *Cyclocarya paliurus* was chemically modified by CSA-Pyr method. Polysaccharides with higher sulfation content showed greater scavenging effect of superoxide radical [1]. Both sulfate and phosphate group are strong electron-withdrawing groups. Most chemical modification of polysaccharides give a greater capacity to donate hydrogen to the superoxide anion and significantly increase their activity for scavenging radicals [14].

##### 4.1.4. Reducing power assay

Reducing properties were generally associated with the presence of reductones, which terminate free radical chain reactions by donating a hydrogen atom [50]. Reductones were also reported to react with certain precursors of peroxide, thus, preventing peroxide formation [72]. Studies also showed that the DS, a stable triple-helical structure, the presence of uronic acid residues and the protein content of a polysaccharide were important factors for reducing the generation of hydroxyl radicals and promoting a polysaccharide's reducing power [1,21,37].

##### 4.2. Anticoagulant activity

Coagulation is an important part of physiological hemostasis. In recent years, cardiovascular diseases, including heart diseases and stroke related to thrombosis, have become the main cause of death globally [37]. Anticoagulants can prevent the formation of thrombus and maintain the health for the patients with cerebral thrombosis, pulmonary embolism and stroke [11].

Heparins are sulfated polysaccharides that have been widely used as clinical anticoagulant and antithrombotic agents. However, they have a number of side effects, including hemorrhage and thrombocytopenia [73]. Some other sulfated polysaccharides have been reported to have anticoagulant and antithrombotic properties and are non-toxic to normal cells [11,37].

Coagulation assays such as activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) are used to evaluate the anticoagulant activities of sulfated polysaccharides and compared to heparin as a reference standard [37].

Sulfated polysaccharides mainly interact ionically through the strong negative charges of their sulfate groups with the positively charged amino acid residues on coagulation factors, such as antithrombin, to exhibit anticoagulant activities [74]. A high DS increases the density of negative charges and enables a sulfated polysaccharide to more strongly neutralize these positively charged amino acid residues [13,40]. Thus, anticoagulant activity is frequently promoted with the increasing of DS. It was reported that sulfated pumpkin polysaccharides exhibits significant anticoagulant activities and shows positive correlation to

**Table 3**  
Bioactivities of chemical modified polysaccharides.

Chemical modification	Bioactivities comparison with of native polysaccharide	Reference
Sulfated modification	Improvement of antioxidant and anticoagulant activities, etc.	[37]
Phosphorylated modification	Stronger anti-tumor and antiviral efficacy, etc.	[42,49]
Carboxymethylated modification	Enhancement effect of antioxidant, etc.	[51]
Selenized modification	Immune-enhancing activity, etc.	[55]
Acetylation modification	Enhancement effect of activation, etc.	[12]
Hydroxypropylation	Higher antioxidant activity, etc.	[18]

Mw over a moderate size range (<7.7 kDa) [13]. In addition to DS and Mw, the patterning of sulfo groups on the polysaccharide chain can also play an important role in anticoagulant activities [40].

#### 4.3. Anti-tumor activity

A large number of studies show that certain polysaccharides and their derivatives exhibit anti-tumor activities by inducing cell apoptosis and arresting the cell cycle [75–77]. The negative charged phosphate group on phosphorylated polysaccharides can bind with receptors on the surface of immune cells with high affinity and activate immune response effectively, leading to produce anti-tumor activities [78]. Long-chain polysaccharides have more opportunities to bind with receptors on the cell than more compact phosphorylated molecules. Thus, chain conformation can play an important role in the anti-tumor activity [43].

Sulfated *Artemisia sphaerocephala* polysaccharide (ASPs) reportedly exhibits anti-tumor activity. In anti-tumor activity assays, the sulfation of PAS significantly improved its capacity to inhibit tumor cells growth by arresting the cell cycle progress in specific phases. Results suggested that the anti-tumor activities of SPAs was not a function of single factor but the result of multiple actions related to many factors, such as DS, MW and PD [19]. Many sulfated polysaccharides can also induce the functional activation of immune cells, such as macrophages, improving their ability of the recognition and reduction of tumor cells [10]. Moreover, some studies indicate that the phosphate group was more beneficial to the enhancement of anti-tumor activity than sulfate or carboxymethyl groups [43].

#### 4.4. Immunoregulatory activity

The immune systems play an important role against pathogen invasion and keeps people healthy. Polysaccharides regulate immunity mainly through two major ways. One is that they can kill the pathogen directly, and the other is that they can strengthen the immune system by enhancing the activities of macrophages and T lymphocytes [11].

Recent studies have shown that sulfation can improve the role of polysaccharides on the phagocytosis of macrophages, promoting the secretion of nitric oxide (NO), reactive oxygen species (ROS), and several cytokines such as tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-1 $\beta$ , IL-6 by macrophages [10]. Sulfated polysaccharides from *Ganoderma atrum* (S-PSG) having different DS were found to have different immunoregulatory activity. The proper DS of sulfo groups is important in immunomodulatory activity, as DS is related to other parameters including Mw, carbohydrate content, sulfation position and glycosidic branching [72]. Selenization can significantly enhance immunoregulatory activity of lily polysaccharides, and it depends on the appropriate ratio between selenium content and carbohydrate content [55]. The acetylated polysaccharide (Ac-CP) from *C. paliurus* shows that acetylation can significantly promote murine macrophages to secrete IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , but also can promote the synthesis of NO and NO synthase in murine macrophage, leading to enhanced immunoregulatory activity [12].

#### 4.5. Anti-viral activity

Polysaccharides can have antiviral activities, and some chemical modification of natural polysaccharides can significantly enhance these antiviral activities [41].

Polysaccharides can exert antiviral activity mainly through the direct killing virus, inhibition of virus adsorption, inhibition of virus invasion, inhibition of viral replication, and by activating the immune system against viral infection [41]. Phosphorylated *Radix Cyathulae officinalis* Kuan polysaccharides (pRCPS) show antiviral activities that are related to their level of phosphor group content. The antiviral mechanism of pRCPS was shown to result from a stable virion- pRCPS complex, which occupies sites on the viral envelop required for viral infection, and

then, preventing CPV adopt to host cells' surface [49]. Phosphorylated *Codonopsis pilosula* polysaccharide has been found to inhibit the virulence of duck hepatitis A virus [79].

#### 4.6. Antibacterial activities

Foods are susceptible to bacterial contamination during processing, storage and transportation, which can result in food poisoning. Previous reports suggested that polysaccharides have anti-biofilm properties, and that polysaccharides can inhibit bacterial growth by blocking the input of nutrients. In the case of sulfated polysaccharides, their antibacterial activity might also be related to the bacteriostatic action of sulfo groups [80].

Rare earths are used to form rare earth–polysaccharide complexes with polysaccharides through chelating coordination of carboxyl groups. It was reported that different types of polysaccharides from different natural sources have a significant impact on antifungal activities of rare earth–carboxymethylated polysaccharide complexes. Also, these polysaccharides can play a key role in promoting cytotoxicity of rare earth complexes towards tested fungi. The rare earth complexes exert various and selective inhibitions against the plant pathogenic fungi [81]. Hydroxaminated and carboxymethylated polysaccharides all showed antibacterial activity, and the inhibitory effects of these modified polysaccharides on gram-positive bacteria were stronger than those on gram negative bacteria [82]. Previous studies showed that carboxymethylated polysaccharides from *Catathelasma ventricosum*, with appropriate DS and stable triple-helical structures, had greater antibacterial activities [50].

#### 4.7. Others

A phosphorylated polysaccharide (PTPP-1) prepared from *Trichosanthes peel* has a significant antiaging activity on the D-galactose-induced aging mice and this anti-aging activity might be related to its antioxidation and immunoregulation abilities [30]. Carboxymethylation of polysaccharide is superior to sulfonation with respect to the in vitro hypoglycemic activities of polysaccharides from *Lachnum* sp., and these bioactivities were affected by the types of substitution groups and their DS [32]. Carboxymethylated polysaccharides from *Morchella angusticeps* Peck (PMEP) showed a stronger cholesterol-lowering activity than PMEP [83]. Selenium modified polysaccharides from *Lachnum* sp. have the activity to reduce the serum and liver lipids, atherogenic index, and enhance the activities of antioxidant enzymes of hyperlipidemic mice, as well improved the histopathological status of hepatic tissues. These may be considered as novel compounds for the treatment of hyperlipidemia and also act as a hepatoprotective agent [33].

## 5. Conclusions

Previous studies have shown that chemical modification of polysaccharides can improve their biological properties and sometimes produce new functional activities. In addition, bioactivities of polysaccharides depend on its molecular structure and other physicochemical properties including water solubility, molecular weight (Mw), degree of substitution (DS), monosaccharide composition, glycosidic bond of the main chain, degree of branching and conformation of the main chains. Although in recent years, the studies in the modification, structure and biological activities of polysaccharides have made a great progress, the mechanism on bioactivities of polysaccharides still are not clearly understood. There is a need to further explore the structure-activity relationship of polysaccharides to obtain new products with desirable functionality attributes.

## Acknowledgements

The study was supported by Natural Science Foundation of Zhejiang Province No. LY17C200017 and National Natural Science Foundation of China No. 31571759.

## References

- [1] J.H. Xie, Z.J. Wang, M.Y. Shen, S.P. Nie, B. Gong, H.S. Li, et al., Sulfated modification, characterization and antioxidant activities of polysaccharide from *Cyclocarya paliurus*[[J]], *Food Hydrocolloid*. 53 (2016) 7–15.
- [2] C. Thetsrimuang, S. Khammuang, K. Chiablaem, C. Srisomsap, R. Sarnthima, Antioxidant properties and cytotoxicity of crude polysaccharides from *Lentinus polychrous* Lévl[[J]], *Food Chem*. 128 (3) (2011) 634–639.
- [3] Y.J. Sun, S.T. Hou, S. Song, B. Zhang, C.Q. Ai, X.F. Chen, et al., Impact of acidic, water and alkaline extraction on structural features, antioxidant activities of *Laminaria japonica* polysaccharides[[J]], *Int. J. Biol. Macromol*. 112 (2018) 985–995.
- [4] Q.L. Tang, G.L. Huang, Preparation and antioxidant activities of cuarbit polysaccharide[[J]], *Int. J. Biol. Macromol*. 117 (2018) 362–365.
- [5] J.L. Wang, A.J. Bao, X.H. Meng, H.Y. Guo, Y.D. Zhang, Y.L. Zhao, et al., An efficient approach to prepare sulfated polysaccharide and evaluation of anti-tumor activities in vitro[[J]], *Carbohydr. Polym*. 184 (2018) 366–375.
- [6] X.M. Wang, Z.S. Zhang, Z.Y. Yao, M.X. Zhao, H.M. Qi, Sulfation, anticoagulant and antioxidant activities of polysaccharide from green algae *Enteromorpha linza*[[J]], *Int. J. Biol. Macromol*. 58 (2013) 225–230.
- [7] T.H. Yang, M. Jia, S.Y. Zhou, F. Pan, Q.B. Mei, Antiviral and immune enhancement activities of sulfated polysaccharide from *Angelica sinensis*[[J]], *Int. J. Biol. Macromol*. 50 (3) (2012) 768–772.
- [8] L.M. Zhao, Y.L. Jia, M. Ma, Y.Q. Duan, L.H. Liu, Prevention effects of Schisandra polysaccharide on radiation-induced immune system dysfunction[[J]], *Int. J. Biol. Macromol*. 76 (3) (2015) 63–69.
- [9] J.H. Xie, X. Liu, M.Y. Shen, S.P. Nie, H. Zhang, C. Li, et al., Purification, physicochemical characterization and anticancer activity of a polysaccharide from *Cyclocarya paliurus* leaves[[J]], *Food Chem*. 136 (3–4) (2013) 1453–1460.
- [10] J. Jiang, F.Y. Meng, Z. He, Y.L. Ning, X.H. Li, H. Song, et al., Sulfated modification of *longan* polysaccharide and its immunomodulatory and antitumor activity in vitro, *Int. J. Biol. Macromol*. 67 (6) (2014) 323–329.
- [11] Z.J. Wang, J.H. Xie, M.Y. Shen, S.P. Nie, M.Y. Xie, Sulfated modification of polysaccharides: synthesis, characterization and bioactivities[[J]], *Trends Food Sci. Tech*. 74 (2018) 147–157.
- [12] X. Liu, J.H. Xie, S. Jia, L.X. Huang, Z.J. Wang, C. Li, et al., Immunomodulatory effects of an acetylated *Cyclocarya paliurus* polysaccharide on murine macrophages RAW264.7[[J]], *Int. J. Biol. Macromol*. 98 (2017) 576–581.
- [13] L. Liang, L. Ao, T. Ma, Y.Y. Ni, X.J. Liao, X.S. Hu, et al., Sulfated modification and anticoagulant activity of pumpkin (*Cucurbita pepo*, Lady Godiva) polysaccharide[[J]], *Int. J. Biol. Macromol*. 106 (2018) 447–455.
- [14] X.M. Wang, Z.S. Zhang, Q. Yao, M.X. Zhao, H.M. Qi, Phosphorylation of low-molecular-weight polysaccharide from *Enteromorpha linza* with antioxidant activity[[J]], *Carbohydr. Polym*. 96 (2) (2013) 371–375.
- [15] X.D. Shi, O.Y. Li, J.Y. Yin, S.P. Nie, Structure identification of  $\alpha$ -glucans from *Dictyophora echinovolata* by methylation and 1D/2D NMR spectroscopy[[J]], *Food Chem*. 271 (2019) 338–344.
- [16] Y.Z. Tao, R.Q. Zhang, W. Yang, H.T. Liu, H.J. Yang, Q.H. Zhao, Carboxymethylated hyperbranched polysaccharide: synthesis, solution properties, and fabrication of hydrogel[[J]], *Carbohydr. Polym*. 128 (2015) 179–187.
- [17] J.H. Xie, F. Zhang, Z.J. Wang, M.Y. Shen, S.P. Nie, M.Y. Xie, Preparation, characterization and antioxidant activities of acetylated polysaccharides from *Cyclocarya paliurus* leaves[[J]], *Carbohydr. Polym*. 133 (2015) 596–604.
- [18] W. Liu, J. Xu, P. Jing, W.B. Yao, X.D. Gao, L.L. Yu, Preparation of a hydroxypropyl *Ganoderma lucidum* polysaccharide and its physicochemical properties[[J]], *Food Chem*. 122 (4) (2010) 965–971.
- [19] J.L. Wang, X.P. Yang, A.J. Bao, X.L. Liu, J.Y. Zeng, X.R. Liu, et al., Microwave-assisted synthesis, structure and anti-tumor activity of selenized *Artemisia sphaerocephala* polysaccharide[[J]], *Int. J. Biol. Macromol*. 95 (2017) 1108–1118.
- [20] X.T. Meng, K.J. Edgar, “Click” reactions in polysaccharide modification[[J]], *Prog. Polym. Sci*. 53 (2016) 52–85.
- [21] J.L. Wang, S.F. Niu, B.T. Zhao, T. Luo, D. Liu, J. Zhang, Catalytic synthesis of sulfated polysaccharides. II: comparative studies of solution conformation and antioxidant activities, *Carbohydr. Polym*. 107 (1) (2014) 221–231.
- [22] M. Bishnoi, A. Jain, P. Hurkat, S.K. Jain, Chondroitin sulphate: a focus on osteoarthritis[[J]], *Glycoconj. J*. 33 (5) (2016) 693–705.
- [23] I. Cumpstey, Chemical modification of polysaccharides, *ISRN Org. Chem*. 27 (2013).
- [24] H.A. Pawar, S.R. Kamat, P.D. Choudhary, An overview of natural polysaccharides as biological macromolecules: their chemical modifications and pharmaceutical applications[[J]], *Biol. Med*. 7 (2015) 224.
- [25] S.J. Li, Q.P. Xiong, X.P. Lai, X. Li, M.J. Wan, J.N. Zhang, et al., Molecular modification of polysaccharides and resulting bioactivities[[J]], *Compr. Rev. Food. Sci. F*. 15 (2) (2016) 237–250.
- [26] Y.F. Xu, S. Song, Y.X. Wei, F.X. Wang, M. Zhao, J. Guo, et al., Sulfated modification of the polysaccharide from *Sphallerocarpus gracilis* and its antioxidant activities[[J]], *Int. J. Biol. Macromol*. 87 (2016) 180–190.
- [27] Y.J. Sun, W. Sun, J.T. Guo, X.T. Hu, G.P. Gong, L.J. Huang, et al., Sulphation pattern analysis of chemically sulphated polysaccharide LbGp1 from *Lycium barbarum* by GC-MS[[J]], *Food Chem*. 170 (2015) 22–29.
- [28] C. Deng, H. Fu, J.J. Xu, J.Y. Shang, Y.M. Cheng, Physicochemical and biological properties of phosphorylated polysaccharides from *Dictyophora indusiata*[[J]], *Int. J. Biol. Macromol*. 72 (2015) 894–899.
- [29] Y. Song, Y.Y. Ni, X.S. Hu, Q.H. Li, Effect of phosphorylation on antioxidant activities of pumpkin (*Cucurbita pepo*, Lady godiva) polysaccharide[[J]], *Int. J. Biol. Macromol*. 81 (2015) 41–48.
- [30] M. Zhang, N.N. Su, Q.L. Huang, Q. Zhang, Y.F. Wang, J.L. Li, et al., Phosphorylation and antiaging activity of polysaccharide from *Trichosanthes peel*[[J]], *J. Food Drug Anal*. 25 (4) (2017) 976–983.
- [31] F. Chen, G.L. Huang, Preparation and immunological activity of polysaccharides and their derivatives[[J]], *Int. J. Biol. Macromol*. 112 (2018) 211–216.
- [32] Y.F. Wang, G.H. Hou, J.L. Li, M.M. Surhio, M. Ye, Structure characterization, modification through carboxymethylation and sulfation, and *in vitro* antioxidant and hypoglycemic activities of a polysaccharide from *Lachnum sp.*[[J]], *Process Biochem*. 72 (2018) 177–187.
- [33] M.M. Surhio, Y. Wang, P. Xu, S. Faisal, J.L. Li, M. Ye, Antihyperlipidemic and hepatoprotective properties of selenium modified polysaccharide from *Lachnum sp.* *Int. J. Biol. Macromol*. 99 (2017) 88–95.
- [34] J.J. Li, X.Z. Hu, X.P. Li, Z. Ma, Effects of acetylation on the emulsifying properties of *Artemisia sphaerocephala* Krasch. polysaccharide[[J]], *Carbohydr. Polym*. 144 (2016) 531–540.
- [35] H.L. Liu, X.L. Liu, Y. Lin, Q.X. Jiang, W.S. Xia, Synthesis, characterization and bioactivities of N,O-carboxylated chitosan, *Int. J. Biol. Macromol*. 91 (2016) 220–226.
- [36] J.L. Wang, A.J. Bao, X.H. Meng, H.Y. Guo, Y.D. Zhang, Y.L. Zhao, et al., An efficient approach to prepare sulfated polysaccharide and evaluation of anti-tumor activities in vitro[[J]], *Carbohydr. Polym*. 184 (2018) 366–375.
- [37] Y.T. Liu, Q.Q. Tang, X.Y. Duan, T.T. Tang, Y. Ke, L. Zhang, et al., Antioxidant and anticoagulant activities of mycelia polysaccharides from *Catathelasma ventricosum* after sulfated modification[[J]], *Ind. Crop. Prod*. 112 (2018) 53–60.
- [38] J.L. Wang, W. Yang, T. Yang, X.N. Zhang, Y. Zuo, J. Tian, et al., Catalytic synthesis of sulfated polysaccharides I: characterization of chemical structure[[J]], *Int. J. Biol. Macromol*. 74 (2015) 61–67.
- [39] Z.Y. Zhu, Y. Liu, C.L. Si, J. Yuan, Q. Lv, Y.Y. Li, et al., Sulfated modification of the polysaccharide from *Cordyceps gunnii* mycelia and its biological activities[[J]], *Carbohydr. Polym*. 92 (1) (2013) 872–876.
- [40] J.H. Yang, K. Luo, D.L. Li, S.S. Yu, J. Cai, L.Y. Chen, et al., Preparation, characterization and *in vitro* anticoagulant activity of highly sulfated chitosan[[J]], *Int. J. Biol. Macromol*. 52 (1) (2013) 25–31.
- [41] L. Chen, G.L. Huang, The antiviral activity of polysaccharides and their derivatives[[J]], *Int. J. Biol. Macromol*. 115 (2018) 77–82.
- [42] Q.L. Huang, L.N. Zhang, Preparation, chain conformation and anti-tumor activities of water-soluble phosphated (1→3)- $\alpha$ -D-glucan from *Poria cocos* mycelia[[J]], *Carbohydr. Polym*. 83 (2011) 1363–1369.
- [43] X.Y. Chen, X.J. Xu, L.N. Zhang, F.B. Zeng, Chain conformation and anti-tumor activities of phosphorylated (1→3)- $\beta$ -D-glucan from *Poria cocos*[[J]], *Carbohydr. Polym*. 78 (3) (2009) 581–587.
- [44] J.L. Wang, Y.X. Wang, L. Xu, Q.Q. Wu, Q. Wang, W.B. Kong, et al., Synthesis and structural features of phosphorylated *Artemisia sphaerocephala* polysaccharide[[J]], *Carbohydr. Polym*. 181 (2018) 19–26.
- [45] X.L. Li, X.F. Tu, T. Kiran, Y.S. Zhang, D.Y. Zhu, J.G. Zhang, et al., Effects of different chemical modifications on the antioxidant activities of polysaccharides sequentially extracted from peony seed dreg[[J]], *Int. J. Biol. Macromol*. 112 (2018) 675–685.
- [46] G.L. Huang, H.L. Huang, The derivatization and antitumor mechanisms of polysaccharides[[J]], *Future Med. Chem*. 9 (16) (2017) 1931–1938.
- [47] L. Ghimici, D.M. Suflet, Phosphorylated polysaccharide derivatives as efficient separation agents for zinc and ferric oxides particles from water[[J]], *Sep. Purif. Technol*. 144 (2015) 31–36.
- [48] M.R.P. Rao, D.U. Warriar, S.R. Gaikwad, P.M. Shevate, Phosphorylation of psyllium seed polysaccharide and its characterization[[J]], *Int. J. Biol. Macromol*. 85 (6) (2016) 317–326.
- [49] H.B. Feng, J. Fan, S.P. Yang, X.L. Zhao, X. Yi, Antiviral activity of phosphorylated *Radix Cyathulae officinalis* polysaccharide against Canine Parvovirus *in vitro*[[J]], *Int. J. Biol. Macromol*. 99 (2017) 511–518.
- [50] Y.T. Liu, X.Y. You, Y.W. Li, L. Zhang, T.T. Tang, X.Y. Duan, et al., Characterization of carboxymethylated polysaccharides from *Catathelasma ventricosum* and their antioxidant and antibacterial activities[[J]], *J. Funct. Foods* 38 (2017) 355–362.
- [51] M.J. Shi, X. Wei, J. Xu, B.J. Chen, D.Y. Zhao, S. Cui, et al., Carboxymethylated degraded polysaccharides from *Enteromorpha prolifera*: preparation and *in vitro*, antioxidant activity[[J]], *Food Chem*. 215 (2017) 76–83.
- [52] X.M. Wang, Z.S. Zhang, M.X. Zhao, Carboxymethylation of polysaccharides from *Tremella fuciformis* for antioxidant and moisture-preserving activities[[J]], *Int. J. Biol. Macromol*. 72 (2) (2015) 526–530.
- [53] G.M. Ren, K.K. Li, Y. Hu, M. Yu, J.J. Qu, X.H. Xu, Optimization of selenizing conditions for Seleno-Lentinan and its characteristics[[J]], *Int. J. Biol. Macromol*. 81 (2015) 249–258.
- [54] Z.Z. Gao, C. Zhang, W.J. Tian, K.H. Liu, R.R. Hou, C.J. Yue, The antioxidative and hepatoprotective effects comparison of Chinese angelica polysaccharide (CAP) and selenizing CAP (sCAP) in CCl<sub>4</sub> induced hepatic injury mice[[J]], *Int. J. Biol. Macromol*. 97 (2017) 46–54.
- [55] R.R. Hou, J. Chen, C.J. Yue, X.P. Li, J. Liu, Z.Z. Gao, et al., Modification of lily polysaccharide by selenylation and the immune-enhancing activity[[J]], *Carbohydr. Polym*. 142 (2016) 73–81.
- [56] C.J. Yue, J. Chen, R.R. Hou, W.J. Tian, K.H. Liu, D.Y. Wang, et al., The antioxidant action and mechanism of selenizing *Schisandra chinensis* polysaccharide in chicken embryo hepatocyte[[J]], *Int. J. Biol. Macromol*. 98 (2017) 506–514.
- [57] Z.Z. Gao, K.H. Liu, W.J. Tian, H.C. Wang, Z.G. Liu, Y.Y. Li, et al., Effects of selenizing angelica polysaccharide and selenizing garlic polysaccharide on immune function of murine peritoneal macrophage[[J]], *Int. Immunopharmacol*. 27 (1) (2015) 104–109.
- [58] T. Qin, Z. Ren, Y.F. Huang, Y.L. Song, D.D. Lin, J. Li, et al., Selenizing *Hericium erinaceus* polysaccharides induces dendritic cells maturation through MAPK and NF- $\kappa$ B signaling pathways[[J]], *Int. J. Biol. Macromol*. 97 (2017) 287–298.

- [59] D.D. Liu, J. Xu, G. Qian, M. Hamid, F. Gan, X.X. Chen, et al., Selenizing astragalus polysaccharide attenuates PCV2 replication promotion caused by oxidative stress through autophagy inhibition via PI3K/AKT activation[J], *Int. J. Biol. Macromol.* 108 (2018) 350–359.
- [60] Z.Y. Zhu, F. Liu, H. Gao, H.Q. Sun, M. Meng, Y.M. Zhang, Synthesis, characterization and antioxidant activity of selenium polysaccharide from *Cordyceps militaris*[J], *Int. J. Biol. Macromol.* 93 (2016) 1090–1099.
- [61] Z.Z. Gao, J. Chen, S.L. Qiu, Y.Y. Li, D.Y. Wang, C. Liu, et al., Optimization of selenylation modification for garlic polysaccharide based on immune-enhancing activity[J], *Carbohydr. Polym.* 136 (7) (2016) 560–569.
- [62] V. Singh, A. Tiwari, D.N. Tripathi, T. Malviya, Microwave promoted methylation of plant polysaccharides[J], *Tetrahedron Lett.* 44 (39) (2003) 7295–7297.
- [63] J.S. Kim, B.L. Reuhs, F. Michon, R.E. Kaiser, R.G. Arumugham, Addition of glycerol for improved methylation linkage analysis of polysaccharides[J], *Carbohydr. Res.* 341 (8) (2006) 1061–1064.
- [64] I.M. Sims, S.M. Carnachan, T.J. Bell, S.F. Hinkley, Methylation analysis of polysaccharides: technical advice[J], *Carbohydr. Polym.* 188 (2018) 1–7.
- [65] Z.S. Zhang, X.M. Wang, M.X. Zhao, H.M. Qi, O-acetylation of low-molecular-weight polysaccharide from *Enteromorpha linza* with antioxidant activity[J], *Int. J. Biol. Macromol.* 69 (2014) 39–45.
- [66] Y. Song, Y. Yang, Y.Y. Zhang, L.S. Duan, C.L. Zhou, Y.Y. Ni, et al., Effect of acetylation on antioxidant and cytoprotective activity of polysaccharides isolated from pumpkin (*Cucurbita pepo*, lady godiva)[J], *Carbohydr. Polym.* 98 (1) (2013) 686–691.
- [67] O. Garcia-Valdez, P. Champagne, M.F. Cunningham, Graft modification of natural polysaccharides via reversible deactivation radical polymerization[J], *Prog. Polym. Sci.* 76 (2018) 151–173.
- [68] J.H. Xie, F. Zhang, Z.J. Wang, M.Y. Shen, S.P. Nie, M.Y. Xie, Preparation, characterization and antioxidant activities of acetylated polysaccharides from *Cyclocarya paliurus* leaves[J], *Carbohydr. Polym.* 133 (2015) 596–604.
- [69] T.T. Hu, D. Liu, Y. Chen, J. Wu, S.S. Wang, Antioxidant activity of sulfated polysaccharide fractions extracted from *Undaria pinnatifida* in vitro[J], *Int. J. Biol. Macromol.* 46 (2) (2010) 193–198.
- [70] J. Wang, Y.M. Zhao, W. Li, Z.B. Wang, L.X. Shen, Optimization of polysaccharides extraction from *Tricholoma mongolicum* Imai and their antioxidant and antiproliferative activities[J], *Carbohydr. Polym.* 131 (2015) 322–330.
- [71] J.I. Ueda, N. Saito, Y. Shimazu, T. Ozawa, Oxidative DNA strand scission induced by copper (II)-complexes and ascorbic acid[J], *Arch. Biochem. Biophys.* 333 (1996) 377–384.
- [72] Y. Chen, H. Zhang, Y.X. Wang, S.P. Nie, C. Li, M.Y. Xie, Sulfated modification of the polysaccharides from *Ganoderma atrum* and their antioxidant and immunomodulating activities, *Food Chem.* 186 (3) (2015) 231–238.
- [73] F. Fabris, G. Luzzatto, P.M. Stefanl, Heparin-induced thrombocytopenia[J], *Haematologica* 85 (2000) 72–81.
- [74] L.H. Fan, L. Jiang, Y.M. Xu, Y. Zhou, Y. Shen, W.G. Xie, et al., Synthesis and anticoagulant activity of sodium alginate sulfates[J], *Carbohydr. Polym.* 83 (4) (2011) 1797–1803.
- [75] M.S. Kokoulin, A.S. Kuzmich, A.I. Kalinovsky, S.V. Tomshich, L. Romanenko, V.V. Mikhailov, et al., Structure and anticancer activity of sulfated O-polysaccharide from marine bacterium *Cobetia litoralis* KMM 3880<sup>T</sup>[J], *Carbohydr. Polym.* 154 (2016) 55–61.
- [76] X.F. Xu, J.G. Yang, Z. Luo, X.W. Zhang, Lentinula edodes-derived polysaccharide enhances systemic and mucosal immunity by spatial modulation of intestinal gene expression in mice[J], *Food Funct.* 6 (6) (2015) 2068–2080.
- [77] S. Zhong, D.F. Ji, Y.G. Li, T.B. Lin, Z.Q. Lv, H.P. Chen, Activation of P27kip1-cyclin D1/E-Cdk2 pathway by polysaccharide from *Phellinus linteus* leads to S-phase arrest in HT-29 cells[J], *Chem. Biol. Interact.* 206 (2) (2013) 222–229.
- [78] P.J. Rice, J.L. Kelley, G. Kogan, H.E. Ensley, J.H. Kalbflesich, I.W. Browder, et al., Human monocyte scavenger receptors are pattern recognition receptors for (1→3)-β-D-glucans, *J. Leukoc. Biol.* 72 (1) (2002) 140–146.
- [79] K. Ming, Y. Chen, F.K. Yao, J.T. Shi, J.J. Yang, H.X. Du, et al., Phosphorylated *Codonopsis pilosula* polysaccharide could inhibit the virulence of duck hepatitis A virus compared with *Codonopsis pilosula* polysaccharide[J], *Int. J. Biol. Macromol.* 94 (2017) 28–35.
- [80] Y.L. Feng, W.Q. Li, X.Q. Wu, L. He, S.Y. Ma, Rapid and efficient microwave-assisted sulfate modification of lentinan and its antioxidant and antiproliferative activities in vitro[J], *Carbohydr. Polym.* 82 (3) (2010) 605–612.
- [81] X.B. Sun, X.Z. Jin, W. Pan, J.P. Wang, Syntheses of new rare earth complexes with carboxymethylated polysaccharides and evaluation of their in vitro antifungal activities, *Carbohydr. Polym.* 113 (113) (2014) 194–199.
- [82] Y.T. Li, B.J. Chen, W.D. Wu, K. Ge, X.Y. Wei, L.M. Kong, et al., Antioxidant and antimicrobial evaluation of carboxymethylated and hydroxamated degraded polysaccharides from *Sargassum fusiforme*[J], *Int. J. Biol. Macromol.* 118 (Part B) (2018) 1550–1557.
- [83] Y. Li, Y. Yuan, L. Lei, F.H. Li, Y.Q. Zhang, J.L. Chen, et al., Carboxymethylation of polysaccharide from *Morchella angusticeps* Peck enhances its cholesterol-lowering activity in rats[J], *Carbohydr. Polym.* 172 (2017) 85–92.