# Research Status and Progress of Polysaccharide Extraction and Antioxidant Activity Evaluation Methods: Postprint

**Authors:** Liu Song, Dong Xiaofang, Tong Jianming

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## Abstract

As one of the four fundamental substances constituting life activities, polysaccharides are closely related to various physiological functions required for sustaining life. With the advancement of research, the antioxidant activity of polysaccharides has become a hot topic of interest for numerous scholars. This paper reviews the current status and progress of research on extraction methods and antioxidant activity evaluation methods for polysaccharides, aiming to provide reference for research on polysaccharide extraction and antioxidant activity evaluation.

## Full Text

### Research Status and Progress of Extraction and Antioxidant Activity Evaluation Methods of Polysaccharides

**LIU Song, DONG Xiaofang*, TONG Jianming**
*(Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China)*

#### Abstract

As one of the four fundamental substances constituting life activities, polysaccharides are closely related to various physiological functions required to maintain life. With the advancement of research, the antioxidant activity of polysaccharides has become a hotspot of interest among scholars. This paper reviews the research status and progress of polysaccharide extraction methods and antioxidant activity evaluation methods, aiming to provide a reference for research on polysaccharide extraction and antioxidant activity evaluation.

**Keywords:** polysaccharides; extraction; antioxidant activity evaluation

Polysaccharides are natural macromolecular compounds widely present in nature, generally composed of more than 10 monosaccharide molecules linearly or branchingly connected through $\alpha$- or $\beta$-glycosidic bonds. As one of the four fundamental substances constituting life activities, polysaccharides are closely related to various physiological functions required to maintain life. Since the 1950s, the functions of polysaccharides and their complexes in cell recognition, differentiation, metabolism, immune response, carcinogenesis, apoptosis, and antioxidant activity have gradually attracted attention. Antioxidant activity represents one of the most important biological activities of polysaccharides, and current research on polysaccharide extraction, antioxidant activity, and their mechanisms of action is becoming increasingly extensive and in-depth.

## 1. Extraction Methods of Polysaccharides

Commonly used polysaccharide extraction methods mainly include water extraction, ultrasound-assisted extraction, microwave-assisted extraction, and enzyme-assisted extraction.

### 1.1 Water Extraction Method

Water extraction is a commonly used method that leverages the water-soluble nature of polysaccharides through hot water immersion or cold water percolation. Factors affecting extraction efficiency include extraction temperature, solid-liquid ratio, extraction time, and number of extraction cycles. Research on water extraction primarily focuses on optimizing these conditions, typically using orthogonal experimental design or response surface methodology to determine the optimal combination of factors. While this method requires simple conditions and is convenient to apply, it tends to co-extract water-soluble components such as proteins and glycosides, resulting in low polysaccharide purity. Additionally, the method is time-consuming and has relatively low extraction efficiency. Polysaccharides extracted using hot water extraction that have been reported in literature include *Pleurotus tuber-regium* polysaccharides [1-2], apple polysaccharides [3-4], sisal polysaccharides [5], *Ganoderma lucidum* polysaccharides [6-7], *Althaea rosea* seed polysaccharides [8], basidiomycete polysaccharides [9], *Epimedium* polysaccharides [10-11], and *Phellinus linteus* polysaccharides [12].

### 1.2 Ultrasound-Assisted and Microwave-Assisted Extraction Methods

Ultrasound-assisted extraction primarily utilizes the intense cavitation effect, mechanical vibration, disturbance effect, emulsification, diffusion, fragmentation, and stirring action generated by ultrasonic waves to increase the movement frequency and speed of material molecules, creating instantaneous cavitation high temperatures and local high pressure that accelerate polysaccharide dissolution in the solvent. Microwave-assisted extraction mainly employs high-frequency electromagnetic waves to penetrate the extraction medium, causing

water molecules within material cells to absorb microwave energy and rapidly increase internal temperature. Continuous high temperature causes cell expansion and rupture, accelerating the free flow of intracellular polysaccharides. Simultaneously, the electromagnetic field generated by microwaves can accelerate the diffusion rate of polysaccharides toward the extraction solvent interface, shortening the time required for polysaccharide molecules to diffuse from inside the material to the solvent interface, thereby improving extraction efficiency.

The advantages of ultrasound-assisted and microwave-assisted extraction methods lie in their ability to utilize physical effects to accelerate cell wall disruption and polysaccharide leaching, improving extraction efficiency while reducing extraction temperature to maximize polysaccharide quality. However, compared with traditional water extraction, these methods require additional optimization of process parameters including ultrasonic/microwave power, frequency, and treatment time. Moreover, due to their rapid internal-to-external heat transfer, excessive power or prolonged treatment may cause cleavage of polysaccharide glycosidic bonds [13], adversely affecting biological activity. Polysaccharides extracted using these methods that have been reported include *Pleurotus tuber-regium* polysaccharides [2], *Cordyceps gunnii* polysaccharides [14], *Codonopsis pilosula* polysaccharides [15], and *Tremella mesenterica* polysaccharides [16].

### 1.3 Enzyme-Assisted Extraction Method

Enzyme-assisted extraction involves adding one or more active enzymes during polysaccharide extraction to accelerate cell wall disruption and polysaccharide leaching, such as cellulase and pectinase to decompose cell walls or protease to remove protein impurities. This approach can employ either multiple enzymes simultaneously (complex enzyme method) [17-18] or a single enzyme individually (single enzyme method) [19]. The advantages of enzyme-assisted extraction include mild conditions and high efficiency. However, the addition of exogenous enzymes may alter the physicochemical properties of polysaccharides, including monosaccharide composition, molecular weight, structure, and conformation [18], potentially affecting biological activity. Additionally, enzyme costs are relatively high, and extraction conditions (particularly temperature and pH) require careful optimization, limiting the application of this method. Polysaccharides extracted using enzyme-assisted methods that have been reported include alfalfa polysaccharides [20], *Epimedium* polysaccharides [11], *Astragalus membranaceus* polysaccharides [21], and *Lycium barbarum* polysaccharides [22].

### 1.4 Other Extraction Methods

Other polysaccharide extraction methods include alkaline extraction [1,9], high-intensity pulsed electric field-assisted extraction [23], and ultrasound-enzyme synergistic-assisted extraction [1]. Although these methods have been sporadically reported, their practical application remains limited. The advantages and disadvantages of various polysaccharide extraction methods are summarized in Table 1.

## 2. Evaluation Methods for Antioxidant Activity of Polysaccharides

### 2.1 In Vitro Studies

In vitro evaluation of polysaccharide antioxidant activity primarily employs chemical and biological methods to assess free radical scavenging capacity, inhibition of lipid peroxidation, and protection against protein oxidative damage. Due to their simplicity and rapidity, in vitro antioxidant evaluation methods have been widely applied. Different evaluation methods are based on distinct reaction principles, each with inherent limitations, making it insufficient to rely on a single method for evaluating polysaccharide antioxidant activity. Consequently, scholars consistently recommend employing at least two in vitro evaluation methods based on different principles when assessing polysaccharide antioxidant capacity.

#### 2.1.1 In Vitro Chemical Evaluation Methods

**2.1.1.1 Free Radical Scavenging Activity Evaluation** Free radicals possess extremely strong oxidative reactivity and can attack any molecules they encounter through oxidation, causing peroxidation of macromolecules in the body and resulting in irreversible damage. Therefore, the free radical scavenging capacity of polysaccharides represents an important aspect of antioxidant activity evaluation. Commonly used methods include DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging, ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging, reactive oxygen species (ROS) scavenging, and reactive nitrogen species (RNS) scavenging. ROS scavenging evaluation mainly comprises peroxyl radical ($ROO\cdot$), superoxide anion radical ($O_2^{-}\cdot$), and hydroxyl radical ($OH\cdot$) scavenging capacity. RNS scavenging evaluation includes nitric oxide radical ($NO\cdot$) and peroxynitrite ($ONOO^{-}$) scavenging capacity.

Wang et al. [3] and Zhang et al. [5] evaluated the DPPH and hydroxyl radical scavenging activities of alfalfa polysaccharides extracted by complex enzymes and sisal polysaccharides extracted by hot water, respectively, finding that both exhibited dose-dependent scavenging activities against DPPH and hydroxyl radicals. Wang et al. [3] reported that apple polysaccharides showed dose-dependent scavenging activity against both DPPH and ABTS radicals. Cheng et al. [10-11] found that *Epimedium* polysaccharides possessed scavenging activity against DPPH, hydroxyl, and ABTS radicals. Additionally, *Althaea rosea* polysaccharides [8], *Ganoderma lucidum* polysaccharides [24], and apple polysaccharides [25] have been reported to exhibit superoxide anion radical scavenging activity. Furthermore, Klaus et al. [9], Ma et al. [26], Li et al. [27], and Volpi et al. [28] investigated the free radical scavenging capacities of basidiomycete polysaccharides, *Inonotus obliquus* polysaccharides, corn silk polysaccharides, and mulberry polysaccharides using one or several of the aforementioned methods, demonstrating that these polysaccharides all possess certain in vitro free

radical scavenging activities.

**2.1.1.2 Reducing Power Evaluation** Polysaccharides also exhibit excellent reducing capacity, which can be used to indirectly evaluate their antioxidant activity. Major methods applied in polysaccharide antioxidant evaluation include ferric ion ($Fe^{3+}$) reducing power assessment, ferrous ion ($Fe^{2+}$) chelating capacity evaluation, and total reducing power determination. Chen et al. [13] and Ma et al. [26] found that *Epimedium* polysaccharides and *Inonotus obliquus* polysaccharides possessed $Fe^{3+}$ reducing capacity. Tseng et al. [7] discovered that *Ganoderma tsugae* polysaccharides exhibited $Fe^{2+}$ chelating capacity and thus antioxidant activity. Additionally, Tseng et al. [7], Liu et al. [8], Dou et al. [25], and Li et al. [27] demonstrated through total reducing power evaluation that *Ganoderma tsugae* polysaccharides, *Althaea rosea* polysaccharides, apple polysaccharides, and *Ziziphus jujuba* cv. Jinsixiaozao possessed certain reducing capacities.

### 2.1.2 In Vitro Biological Evaluation

**2.1.2.1 Inhibition of Lipid Peroxidation Activity Evaluation** Studies have shown that polysaccharides can chelate metal ions necessary for ROS generation. The alcoholic hydroxyl groups on polysaccharide rings can chelate metal ions such as $Fe^{2+}$ and $Cu^{2+}$ that are required for hydroxyl radical production, preventing hydroxyl radical formation. Since hydroxyl radicals can initiate lipid peroxidation reactions that generate lipid hydroperoxides ($H_2O_2$), polysaccharides can inhibit lipid peroxidation. $Fe^{2+}$/ascorbate treatment of isolated mouse liver can induce lipid peroxidation. Current studies have found that *Inonotus obliquus* polysaccharides [26], seaweed polysaccharides [29], *Ganoderma lucidum* polysaccharides [30], and *Asparagus cochinchinensis* polysaccharides [31] can inhibit $Fe^{2+}$/ascorbate-induced liver lipid peroxidation. Additionally, *Epimedium* polysaccharides [10] and *Tremella mesenterica* polysaccharides [16] have been shown to inhibit erythrocyte membrane lipid peroxidation and protect membrane structural integrity.

**2.1.2.2 Inhibition of Protein Oxidative Damage Activity Evaluation** Protein oxidative damage can cause a series of physiological and pathological disorders [32]. Subramanian et al. [33] found that *Tinospora cordifolia* polysaccharides could inhibit radiation-induced protein damage.

**2.1.3 In Vitro Cell Model Evaluation** Since cell-based systems more closely approximate the in vivo environment, using cells as carriers for preliminary screening and evaluation of antioxidant activity has become a preferable approach in polysaccharide antioxidant research compared to in vitro chemical and biological methods. Researchers can select different model cells according to the intended application of the antioxidant substance to evaluate its antioxidant capacity against specific oxidative stress responses in

organisms [34-40]. Table 2 lists commonly used cell models in polysaccharide antioxidant activity research and their applications.

### 2.2 In Vivo Evaluation

Although in vitro evaluation methods for polysaccharide antioxidant activity are convenient and rapid, the processes of digestion, absorption, and metabolism introduce uncertainties regarding polysaccharide antioxidant capacity. Consequently, results from in vitro studies alone are insufficient for accurate evaluation of antioxidant activity. Currently, using representative oxidative stress animal models to evaluate polysaccharide antioxidant activity has gained broad recognition. When oxidative stress occurs in animals, excessive production of highly reactive molecules such as ROS and RNS overwhelms the scavenging capacity of endogenous antioxidants, creating an imbalance between oxidant and antioxidant systems that leads to tissue damage. During oxidative stress, various cellular components including lipids, carbohydrates, proteins, and nucleic acids (DNA or RNA) undergo varying degrees of oxidation, causing oxidative damage such as denaturation, cross-linking, and fragmentation [34-36], which subsequently results in destruction of cellular structure and function, cell death, tissue injury, organ pathology, and even cancer [37].

Studies have demonstrated that polysaccharide antioxidant activity primarily manifests in protecting against protein damage, lipid peroxidation, and DNA/RNA oxidative damage in organs such as the liver and kidneys where oxidative radicals are concentrated, scavenging free radicals, protecting antioxidant systems (such as antioxidant enzymes), and maintaining normal mitochondrial membrane potential. Commonly used animal models for polysaccharide antioxidant evaluation include systemic oxidative stress models that induce whole-body oxidative stress, such as D-galactose models, transgenic animal models, hyperlipidemia models, fatty acid oxidation stress models, and radiation damage models. Additionally, there are local organ-specific oxidative stress models that target particular tissues or organs, including carbon tetrachloride ($CCl_4$)-induced liver injury models, cyclosporine A-induced liver or kidney injury models, streptozotocin-induced diabetes (pancreatic injury) models, and methionine-choline deficiency liver injury models. Table 3 lists commonly used animal models in polysaccharide antioxidant activity research and their applications.

### 2.3 Common Methods and Indicators for Cell and Animal Model Evaluation

Polysaccharide antioxidant activity evaluation research primarily focuses on analyzing oxidative stress markers, antioxidant enzymes and substances, intracellular calcium levels, mitochondrial membrane potential, and mitochondrial apoptosis. Based on the type of oxidative stress substances, quantitative evaluation methods mainly include detection of protein oxidative damage markers, lipid peroxidation markers, DNA or RNA damage, and antioxidant enzyme

systems and substances. Studies have demonstrated that polysaccharides exhibit promising antioxidant activity in alleviating oxidative damage to proteins, lipids, and nucleic acids, regulating antioxidant enzyme and substance levels, and inhibiting apoptosis.

**2.3.1 Protein Oxidative Damage Analysis** During oxidative stress, free radical damage to proteins includes peptide chain breakage, intermolecular cross-linking and polymerization, oxidative deamination of amino acids, attack on protein reducing groups, and cross-linking between proteins and malondialdehyde (MDA) generated from lipid oxidation. Current detection indicators for protein oxidative damage primarily include two parameters: protein carbonyl formation (carbonylation) and dityrosine formation (tyrosine nitration) [59-62], with protein carbonyl level measurement being more commonly employed in experimental studies. Josephine et al. [56] found that *Sargassum wightii* polysaccharides could reduce the degree of protein carbonylation and amide formation in cyclosporine A-induced liver injury rats, thereby decreasing protein oxidative damage and alleviating oxidative stress.

**2.3.2 Lipid Peroxidation Analysis** Lipid peroxidation involves ROS reacting with phospholipids in biological membranes, enzymes, polyunsaturated fatty acid side chains of membrane receptors, and macromolecules such as nucleic acids to form lipid peroxidation products including MDA and 4-hydroxynonenal (HNE), which alter cell membrane fluidity and permeability, ultimately leading to changes in cell structure and function [63-64]. HNE and MDA are two highly toxic end products of lipid peroxidation commonly used as indicators, with MDA content measurement being more frequently employed in experimental studies. Through measurement of MDA content, Jia et al. [38] found that *Gynostemma pentaphyllum* polysaccharides could reduce $\beta$-amyloid peptide (A$\beta$)-induced lipid peroxidation levels in PC12 cells; Li et al. [48] demonstrated that *Acanthopanax senticosus* polysaccharides could reduce lipid peroxidation levels in rats with radiation-induced oxidative stress; Yu et al. [50] showed that *Euphorbia kansui* polysaccharides could reduce lipid peroxidation in mice with swimming fatigue-induced oxidative stress; and Josephine et al. [56] found that *Sargassum wightii* polysaccharides could reduce lipid peroxidation in cyclosporine A-induced liver injury rats.

**2.3.3 DNA or RNA Damage Analysis** Free radicals can directly attack biological macromolecules DNA or RNA, inducing oxidative damage [65-66]. The most common lesion is oxidation of the guanine 8-carbon atom to form 8-hydroxy-[deoxy]guanine (8-OHdG/8-OHG). Additionally, direct removal of some purine or pyrimidine bases creates apurinic and apyrimidinic sites, collectively referred to as AP sites. Beyond these oxidative damages, DNA double-strand breaks (DSBs) represent the most dangerous and severe type of DNA damage in cells. Experimental studies commonly employ 8-OHdG content measurement to evaluate DNA damage. The comet assay is also frequently used

for DNA damage evaluation. Also known as single cell gel electrophoresis assay (SCGE), this recently developed sensitive method quantitatively detects DNA damage at the single-cell level. The principle involves fragmented DNA moving out of the nucleus under electric field influence while intact supercoiled DNA remains within the nucleus, creating a "comet tail" phenomenon with intact chromosomal DNA forming the "head" and loose or broken DNA forming the "tail" during gel electrophoresis. The percentage of tail DNA fluorescence correlates with DNA damage severity. Josephine et al. [56] found that *Sargassum wightii* polysaccharides could reduce 8-OHdG content in cyclosporine A-induced liver injury rats, alleviating DNA damage. Tsai et al. [40] demonstrated that *Antrodia cinnamomea* polysaccharides could reduce DNA damage in $H_2O_2$-induced Zhang liver cells by measuring 8-OHdG content, and further confirmed through comet assay that these polysaccharides could reduce $H_2O_2$-induced DNA oxidative damage in Zhang liver cells.

**2.3.4 ROS, Antioxidant Enzymes, and Antioxidant Substances Analysis** Aerobic cells produce various ROS during metabolism, with excessive ROS being harmful to cells. Various active enzymes and antioxidants in cells play crucial roles in defending against oxidative damage and maintaining oxidant-antioxidant balance, such as oxygen radical enzymes converting ROS into less toxic substances [67] and the detoxification function of antioxidant substances [68]. Key antioxidant enzymes include alkaline phosphatase (ALP), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and superoxide dismutase (SOD). SOD is the primary scavenger of superoxide anions, catalyzing their decomposition into $H_2O_2$, which still possesses oxidative damage potential that CAT then converts to oxygen ($O_2$) and water ($H_2O$). Meanwhile, $H_2O_2$ can also react with reduced glutathione (GSH) under GSH-Px catalysis to form $H_2O$ and oxidized glutathione. Additionally, non-enzymatic antioxidant substances such as GSH, vitamin C, and vitamin E also play important roles in maintaining oxidant-antioxidant balance.

Furthermore, polysaccharides can directly quench or inhibit free radicals by serving as hydrogen proton or electron donors, terminating free radical chain reactions. The possible mechanisms include: (1) polysaccharides releasing small, highly reactive hydrogen protons that capture highly reactive free radicals and convert them into inactive or more stable compounds; and (2) direct electron transfer to scavenge free radicals. Numerous studies have found that *Antrodia cinnamomea* polysaccharides [40], *Lycium barbarum* polysaccharides [43], *Acanthopanax senticosus* polysaccharides [48], *Euphorbia kansui* polysaccharides [50], *Sargassum wightii* polysaccharides [56], and *Lycium barbarum* polysaccharides [57] can promote the generation of antioxidant enzymes including GSH-Px, GR, and SOD. Ma et al. [43] found that *Lycium barbarum* polysaccharides could prevent the decline of non-enzymatic antioxidants GSH, vitamin C, and vitamin E in the livers of high-fat diet-induced oxidative stress mice, thereby alleviating oxidative stress. Additionally, Jia et al. [38] found that *Gynostemma pentaphyllum* polysaccharides could reduce ROS content in A$\beta$-induced PC12 cells,

and Tsai et al. [40] confirmed that *Antrodia cinnamomea* polysaccharides could reduce ROS content in $H_2O_2$-induced Zhang liver cells.

**2.3.5 Intracellular Calcium, Mitochondrial Membrane Potential, and Mitochondrial Apoptosis Analysis** Excessive oxidative stress can cause elevated intracellular calcium levels, leading to decreased mitochondrial membrane potential and activation of mitochondrial apoptosis pathways. Through measurement of intracellular calcium levels, mitochondrial membrane potential, and Western blot analysis, Berridge et al. [68] found that *Gynostemma pentaphyllum* polysaccharides could alleviate A$\beta$-induced oxidative damage in PC12 cells by reducing intracellular calcium levels, maintaining normal mitochondrial membrane potential, decreasing Bax/Bcl-2 expression, reducing mitochondrial cytochrome C release, and inhibiting caspase-3 (CPP3) activity [38].

In summary, different polysaccharide extraction methods each have distinct advantages and disadvantages, and the appropriate method should be selected based on practical considerations. Meanwhile, the antioxidant mechanisms of polysaccharides are complex, and evaluation research should combine in vitro and in vivo methods with hierarchical, purposeful investigation to clearly understand polysaccharide antioxidant mechanisms and enable effective extraction and utilization.

## References

[1] WU G H,HU T,HUANG Z L,et al.Characterization of water and alkali-soluble polysaccharides Pleurotus tuber-regium sclerotia[J].Carbohydrate Polymers,2013,96(1):284–290.
[2] ZHANG M,ZHANG L N,CHEUNG P C K,et al.Molecular weight and anti-tumor activity of the water-soluble polysaccharides isolated by hot water and ultrasonic treatment from the sclerotia and mycelia of Pleurotus tuber-regium[J].Carbohydrate Polymers,2004,56(2):123–128.
[3] WANG X,LÜ X.Characterization of pectic polysaccharides extracted from apple pomace by hot-compressed water[J].Carbohydrate Polymers,2014,102:174–184.
[4] YANG X B,YANG S,GUO Y R,et al.Compositional characterisation of soluble apple polysaccharides,and their antioxidant and hepatoprotective effects on acute CCl4-caused liver damage in mice[J].Food Chemistry,2013,138(2/3):1256–1264.
[5] ZHANG X H,LIU L N,LIN C W.Isolation,structural characterization and antioxidant activity of a neutral polysaccharide from Sisal waste[J].Food Hydrocolloids,2014,39:10–18.
[6] LAI L S,YANG D H.Rheological properties of the hot-water extracted polysaccharides in Ling-Zhi (Ganoderma lucidum)[J].Food Hydrocolloids,2007,21(5/6):739–746.
[7] TSENG Y H,YANG J H,MAU J L.Antioxidant properties of polysaccharides from Ganoderma tsugae[J].Food Chemistry,2008,107(2):732–738.

[8] LIU F,LIU W H,TIAN S G.Artificial neural network optimization of Althaea rosea seeds polysaccharides antioxidant activity[J].International Journal of Biological Macromolecules,2014,70:100-107.
[9] KLAUS A,KOZARSKI M,NIKSIC M,et al.Antioxidative activities and chemical characterization of polysaccharides extracted the basidiomycete Schizophyllum commune[J].LWT-Food Science and Technology,2011,44(10):2005-2011.
[10] CHENG H R,FENG S L,SHEN S A,et al.Extraction,antioxidant and antimicrobial activities of Epimedium acuminatum Franch.polysaccharide[J].Carbohydrate Polymers,2013,96(1):101-108.
[11] CHENG H R,FENG S L,JIA X J,et al.Structural characterization and antioxidant activities polysaccharides extracted Epimedium acuminatum[J].Carbohydrate Polymers,2013,92(1):63-68.
[12] WANG Z B,PEI J J,MA H L,et al.Effect of extraction media on preliminary characterizations and antioxidant activities of Phellinus linteus polysaccharides[J].Carbohydrate Polymers,2014,109:49-55.
[13] CHEN R Z,LI S Z,LIU C M,et al.Ultrasound complex enzymes assisted extraction and biochemical activities polysaccharides Epimedium leaves[J].Process Biochemistry,2012,47(12):2040-2050.
[14] ZHU Z Y,PANG W,LI Y Y,et al.Effect of ultrasonic treatment on structure and antitumor activity of mycelial polysaccharides Cordyceps gunnii[J].Carbohydrate Polymers,2014,114:12-20.
[15] ZOU Y F,CHEN X F,YANG W Y,et al.Response surface methodology for optimization of the ultrasonic extraction of polysaccharides from Codonopsis pilosula Nannf. var. modesta L. T. Shen[J].Carbohydrate Polymers,2011,84(1):503-508.
[16] YAN Y L,YU C H,CHEN J,et al.Ultrasonic-assisted extraction optimized by response surface methodology,chemical composition and antioxidant activity of polysaccharides from Tremella mesenterica[J].Carbohydrate Polymers,2011,83(1):217-224.
[17] YIN X L,YOU Q H,JIANG Z H.Optimization of enzyme assisted extraction of polysaccharides from Tricholoma matsutake by response surface methodology[J].Carbohydrate Polymers,2011,86(3):1358-1364.
[18] ZHU Y,LI Q,MAO G H,et al.Optimization of enzyme-assisted extraction and characterization polysaccharides Hericium erinaceus[J].Carbohydrate Polymers,2014,101:606-613.
[19] LI S N,HAN D D,ROW K H,et al.Optimization of enzymatic extraction of polysaccharides from some marine algae by response surface methodology[J].Korean Journal of Chemical Engineering,2012,29(5):650-656.
[20] WANG S P,DONG X F,TONG J M.Optimization of enzyme-assisted extraction of polysaccharides from alfalfa and its antioxidant activity[J].International Journal of Biological Macromolecules,2013,62:387-396.
[21] CHEN H G,ZHOU X,ZHANG J Z.Optimization of enzyme assisted extraction of polysaccharides from Astragalus membranaceus[J].Carbohydrate Polymers,2014,111:567-575.
[22] ZHANG J,JIA S Y,LIU Y,et al.Optimization of enzyme-assisted extraction

of the Lycium barbarum polysaccharides using response surface methodology[J].Carbohydrate Polymers,2011,86(2):1089-1092.
[23] ZHAO W Z,YU Z P,LIU J B,et al.Optimized extraction of polysaccharides from corn silk by pulsed electric field and response surface quadratic design[J].Journal of the Science of Food and Agriculture,2011,91(12):2201-2209.
[24] CHEN X P,CHEN Y,LI S B,et al.Free radical scavenging of Ganoderma lucidum polysaccharides and its effect on antioxidant enzymes and immunity activities in cervical carcinoma rats[J].Carbohydrate Polymers,2009,77(2):389-393.
[25] DOU J,MENG Y H,LIU L,et al.Purification,characterization and antioxidant activities of polysaccharides thinned-young apple[J].International Journal of Biological Macromolecules,2015,72:31-40.
[26] MA L S,CHEN H X,ZHU W C,et al.Effect of different drying methods on physicochemical properties and antioxidant activities of polysaccharides extracted from mushroom Inonotus obliquus[J].Food Research International,2013,50(2):633-640.
[27] LI J W,LIU Y F,FAN L P,et al.Antioxidant activities of polysaccharides from the fruiting bodies of Zizyphus Jujuba cv.Jinsixiaozao[J].Carbohydrate Polymers,2011,84(1):390-394.
[28] VOLPI N,TARUGI P.Influence of chondroitin sulfate charge density,sulfate group position,and molecular mass on Cu2+ -mediated oxidation of human low-density lipoproteins: effect of normal human plasma-derived chondroitin sulfate[J].Journal of Biological Chemistry,1999,125(2):297-304.
[29] DE SOUZA M C R,MARQUES C T,DORE C M G,et al.Antioxidant activities of sulfated polysaccharides brown seaweeds[J].Journal Applied Phycology,2007,19(2):153-160.
[30] LEE J M,KWON H,JEONG H,et al.Inhibition of lipid peroxidation and oxidative DNA damage by Ganoderma lucidum[J].Phytotherapy Research,2001,15(3):245-249.
[31] LI Z X,HUANG C G,CAI Y J,et al.Chemical structure and in vitro antioxidant activity of polysaccharides from Asparagus cochinchinensis[J].Acta Pharmaceutica Sinica,2000,35(5):358-362.
[32] STADTMAN E R,BERLETT B S.Reactive oxygen-mediated protein oxidation in aging and disease[J].Chemical Research in Toxicology,1997,10(5):485-494.
[33] SUBRAMANIAN M,CHINTALWAR G J,CHATTOPADHYAY S.Antioxidant properties of a Tinospora cordifolia polysaccharide against iron-mediated lipid damage and $\gamma$-ray induced protein damage[J].Redox Report,2002,7(3):137-143.
[34] HALLIWELL B,GUTTERIDGE J M C.Free radicals in biology and medicine[M].New York:Oxford University Press,1998:105-107.
[35] DEAN R T,GIESEG S,DAVIES M J.Reactive species and their accumulation on radical damaged proteins[J].Trends in Biochemical Science,1993,18(11):437-441.
[36] ARUOMA O I.Free radicals,oxidative stress,and antioxidants in human health and disease[J].Journal of the American Oil Chemists' Soci-

ety,1998,75(2):199-212.
[37] VALKO M,IZAKOVIC M,MAZUR M,et al.Role of oxygen radicals in DNA damage and cancer incidence[J].Molecular and Cellular Biochemistry,2004,266(1/2):37-56.
[38] JIA D,RAO C G,XUE S X,et al.Purification,characterization and neuroprotective effects of a polysaccharide from Gynostemma pentaphyllum[J].Carbohydrate Polymers,2015,122:93-100.
[39] LI S P,ZHAO K J,JI Z N,et al.A polysaccharide isolated from Cordyceps sinensis,a traditional Chinese medicine,protects PC12 cells against hydrogen peroxide-induced injury[J].Life Sciences,2003,73(19):2503-2513.
[40] TSAI M C,SONG T Y,SHIH P H,et al.Antioxidant properties of water-soluble polysaccharides Antrodia cinnamomea submerged culture[J].Food Chemistry,2007,104(3):1115-1122.
[41] HASSAN S,EL-TWAB S A,HETTA M,et al.Improvement of lipid profile and antioxidant of hypercholesterolemic albino rats by polysaccharides extracted from the green alga Ulva lactuca Linnaeus[J].Saudi Journal of Biological Sciences,2011,18(4):333-340.
[42] ZHAO S,RONG C B,LIU Y,et al.Extraction of a soluble polysaccharide from Auricularia polytricha and evaluation of its anti-hypercholesterolemic effect in rats[J].Carbohydrate Polymers,2015,122:39-45.
[43] MA M,LIU G H,YU Z H,et al.Effect of the Lycium barbarum polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in vivo[J].Food Chemistry,2009,113(4):872-877.
[44] GE X,CHEN T T,CAI J Y,et al.Study on antioxidant activity of polysaccharides from Cyclocarya paliurus[J].Journal of Chinese Institute of Food Science and Technology,2011,11(5):59-64.
[45] QIAO D L,KE C L,HU B,et al.Antioxidant activities of polysaccharides from Hyriopsis cumingii[J].Carbohydrate Polymers,2009,78(2):199-204.
[46] KE C L,QIAO D L,GAN D,et al.Antioxidant acitivity in vitro and in vivo of the capsule polysaccharides Streptococcus subsp.zooepidemicus[J].Carbohydrate Polymers,2009,75(4):677-682.
[47] DANG Y L.Protective effect of Acanthopanax giraldii polysaccharides on experimental liver injury induced by carbon tetrachloride[J].China Journal of Chinese Materia Medica,1997,22(3):176-178.
[48] LI X L,ZHOU A G.Preparation of polysaccharides from Acanthopanax senticosus and its inhibition against irradiation-induced injury of rat[J].Carbohydrate Polymers,2007,67(2):219-226.
[49] WEI W S,TAN J Q,GUO F,et al.Effects of Coriolus versicolor polysaccharides on superoxide dismutase activities in mice[J].Acta Pharmacologica Sinica,1996,17(2):174-178.
[50] YU F R,LU S Q,YU F H,et al.Protective effects of polysaccharide from Euphorbia kansui (Euphorbiaceae) on the swimming exercise-induced oxidative stress in mice[J].Canadian Journal of Physiology and Pharmacology,2006,84(10):1071-1079.
[51] CHI A P,KANG C Z,ZHANG Y,et al.Immunomodulating and antioxidant effects of polysaccharide conjugates from the fruits of Ziziphus Jujube on

Chronic Fatigue Syndrome rats[J].Carbohydrate Polymers,2015,122:189-196.
[52] JIANG C X,XIONG Q P,GAN D,et al.Antioxidant activity and potential hepatoprotective effect of polysaccharides from Cyclina sinensis [J].Carbohydrate Polymers,2013,91(1):262-268.
[53] CHEN C Y,DING Y Q,ELMAHADI E A,et al.Effect of polysaccharides from Indocalamus leaf on experimental liver injury induced by carbon tetrachloride in mice[J].Chinese Journal of Pharmacology and Toxicology,1996,10(3):237-238.
[54] YANG Y,HU X X,ZHOU L L,et al.Protective effect of Solanum nigrum polysaccharides on acute liver injury induced by CCl4 in mice[J].Chinese Traditional Patent Medicine,2014,36(12):2602-2605.
[55] JOSEPHINE A,VEENA C K,AMUDHA G,et al.Evaluating the effect of sulphated polysaccharides on cyclosporine a induced oxidative renal injury[J].Molecular and Cellular Biochemistry,2006,287(1/2):101-108.
[56] JOSEPHINE A,NITHYA K,AMUDHA G,et al.Role of sulphated polysaccharides from Sargassum Wightii in Cyclosporine A-induced oxidative liver injury in rats[J].BMC Pharmacology,2008,8(1):4.
[57] LI X M.Protective effect of Lycium barbarum polysaccharides on streptozotocin-induced oxidative stress rats[J].International Journal Biological Macromolecules,2007,40(5):461-465.
[58] JIA J,ZHANG X,HU Y S,et al.Evaluation of in vivo antioxidant activities of Ganoderma lucidum polysaccharides in STZ-diabetic rats[J].Food Chemistry,2009,115(1):32-36.
[59] DAVIES K J.Protein damage and degradation by oxygen radicals. .General aspects[J].The Journal of Biological Chemistry,1987,262(20):9895-9901.
[60] DAVIES K J,DELSIGNORE M E,LIN S W.Protein damage and degradation by oxygen radicals. .Modification amino acids[J].The Journal Biological Chemistry,1987,262(20):9902-9907.
[61] DAVIES K J,DELSIGNORE M E.Protein damage and degradation by oxygen radicals. .Modification of secondary and tertiary structure[J].The Journal of Biological Chemistry,1987,262(20):9908-9913.
[62] DAVIES K J,LIN S W,PACIFICI R E.Protein damage and degradation by oxygen radicals. .Degradation denatured protein[J].The Journal Biological Chemistry,1987,262(20):9914-9920.
[63] RICE-EVANS C,BURDON R.Free radical-lipid interactions and their pathological consequences[J].Progress in Lipid Research,1993,32(1):71-110.
[64] HALLIWELL B,GUTTERRIDGE J M C.Lipid peroxidation,oxygen radicals,cell damage,and antioxidant therapy[J].The Lancet,1984,323(8391):1396-1397.
[65] IMLAY A,LINN S.DNA damage oxygen radical toxicity[J].Science,1988,240(4857):1302-1309.
[66] COTGREAVE I A,MOLDEUS P,ORRENIUS S.Host biochemical defense mechanisms against prooxidants[J].Annual Review of Pharmacology and Toxicology,1988,28(1):189-212.
[67] ARUOMA O I.Characterization of drugs as antioxidant prophylactics[J].Free Radical Biology and Medicine,1996,20(5):675-705.

[68] BERRIDGE M J,LIPP P,BOOTMAN M D.Signal transduction.The calcium entry pas de deux[J].Science,2000,287(5458):1604-1605.

*Note: Figure translations are in progress. See original paper for figures.*