

Changes in the structure of polysaccharides under different extraction methods

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1 | INTRODUCTION

Polysaccharides are carbohydrates composed of more than 10 polyhydroxy aldehydes or ketones covalently linked through the glycosidic bonds of different monosaccharides, which are found in plants, animals, and microorganisms (H. Huang & Huang, 2020; Y. Liu & Huang, 2019). Polysaccharides have a large molecular weight and complex structure, including the primary and advanced structures, and have stereotyped and amorphous structures. Among them, the primary structure is reflected mainly in the connection mode of the adjacent sugar rings, the composition of monosaccharides and the residue connection sequence, the structure of anomeric carbon, the branching situation of the sugar chain, or the length of the branch chain (G. Huang et al., 2021). The advanced structures include a variety of polymers formed by hydrogen bonding between the leading chains of polysaccharides (related to the conformation of the main chain structure of polysaccharides molecules but does not involve the spatial arrangement of side chains), advanced structures also include new ordered conformations formed by noncovalent bond interactions between sugar units (Rao et al., 2021).

As an essential prerequisite for developing and functional research of the corresponding polysaccharides products, the effective extraction of polysaccharides is an important step, and the number of related articles has been increasing in recent years (Figure 1). The extraction

methods of polysaccharides also have their own advantages and disadvantages (Figure 2). However, different extraction methods of polysaccharides have resulted in differences in the molecular weight, monosaccharide content, and structural composition of the extracted polysaccharides. The extraction conditions can cause hydrolysis of molecular chains and cleavage of intermolecular hydrogen bonds, resulting in polysaccharides with different physicochemical and functional properties (Alboofetileh et al., 2019; Benchamas et al., 2020). The environmental conditions of extraction (such as temperature, pH, solid–liquid ratio, solvent, etc.) and auxiliary extraction process (enzyme, ultrasonic, microwave, etc.) had important effects on the structures and properties of polysaccharides (Figure 3), which are also the essential reasons for the different components and structures of polysaccharides extracted from the same raw materials and methods. Currently, most researchers focus mainly on the analysis and research of the yield or biological activity of polysaccharides using the extraction method, and the study of the structure of polysaccharides is the basis for discussing its bioactivity, which has not been summarized in depth. This manuscript focuses on the effects of different extraction processes, including hot water extraction (HWE), acid (alkaline) extraction, enzyme-assisted extraction (EAE), subcritical water extraction (SWE), ultrasonic extraction technology, and microwave-assisted extraction (MAE) technology, on the structure of polysaccharides. The extraction process of the equipment is shown in Figure 4.

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1.1 | HWE

HWE generally requires a longer extraction time and a higher extraction temperature to improve polysaccharide yield (X. Shi et al., 2023). The polysaccharides extracted by this method usually have a higher molecular weight and a more stable structure, which is beneficial to the precipitation of acidic glycan (P. Liu et al., 2021). However, too high or sustained high temperature can damage the molecular chain to expose reactive groups and cause structural changes, such as molecular degradation or side chain breakage. For example, heat treatment at 150°C for 6 h resulted in a decrease in the molecular weight of β -D-glucan of *Grifola frondosa* from

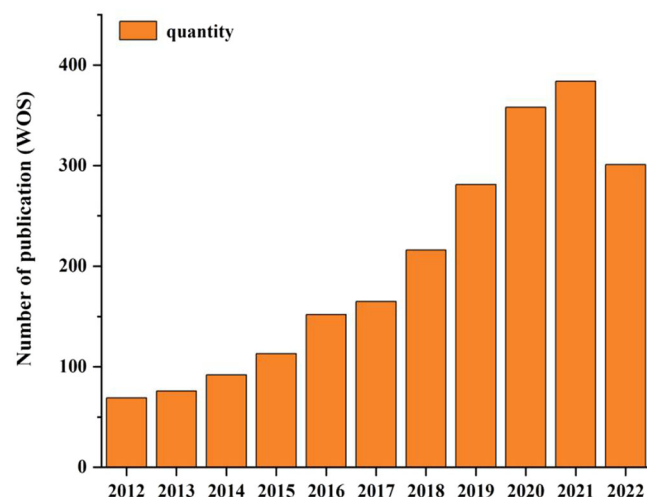


FIGURE 1 Number of articles on extracting polysaccharide structure (Topic: Polysaccharides, Extraction, and Structures. Source: Web of Science data for the past 10 years as of September 24, 2022).

800 to 6.4 kDa, and the structure changed from ordered (99.0%) to disordered (67.5%). The primary structures of fucoidan and chondroitin sulfate remain unchanged below 140°C, while conformational transitions and demolecular reactions occur at higher temperatures (Yi et al., 2020). Under the same extraction time and solid–liquid ratio, the extraction of hot water has higher molecular weight components and root mean square radius of rotation (R_z) than the extraction of *Moringa oleifera* leaf polysaccharides in warm water, the extracted polysaccharides fraction contained more galacturonic acid, the glucose and galactose ratio decreased. The surface of polysaccharides extracted with hot water had irregular bumps after drying, while the surface of polysaccharides extracted with warm water had some tiny pores on the surface (Y. Yang, Zhao, et al., 2020). The *Lentinus edodes* polysaccharides showed higher molecular weight and triple helix signal when the extraction temperature increased from 100°C to 130°C. However, when the temperature is further increased to 150°C, the molecular chain of polysaccharides will be destroyed, resulting in degradation of molecular weight and weakening of the signal. Prolonged high temperatures could degrade polysaccharides into oligosaccharides, monosaccharides, and other by-products, such as 5-Hydroxymethyl-2-furaldehyde, and so on (Yoo et al., 2020).

1.2 | Acid (alkaline) extraction

Unlike HWE, acid and alkali easily break the plant cell wall, destroy the ester bond and the hydrogen bond between polysaccharides and usually aid in the release and dissolution of polysaccharides during extraction, resulting

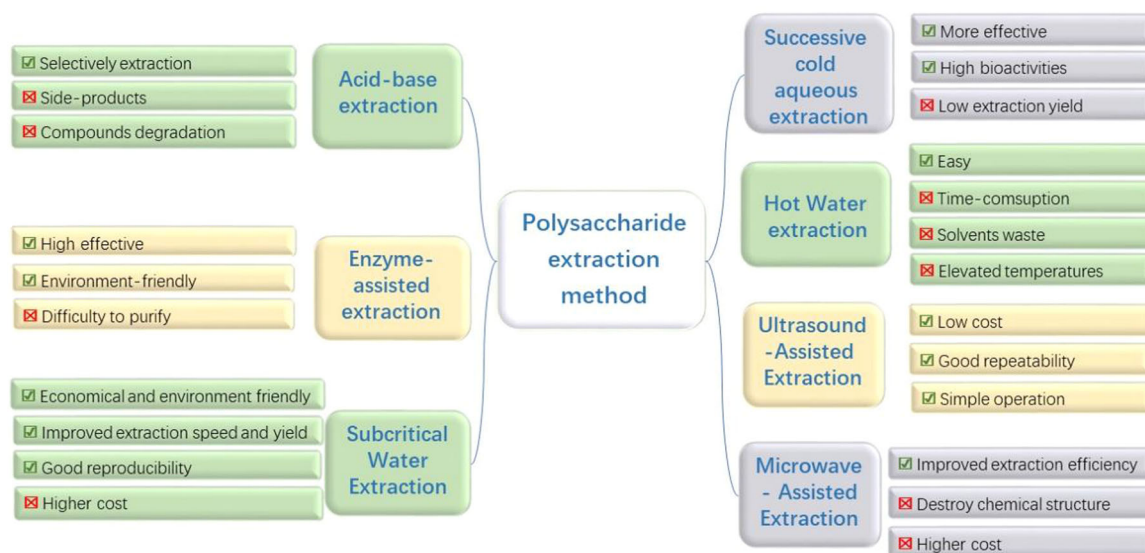


FIGURE 2 Advantages and disadvantages of various extraction methods for polysaccharides.

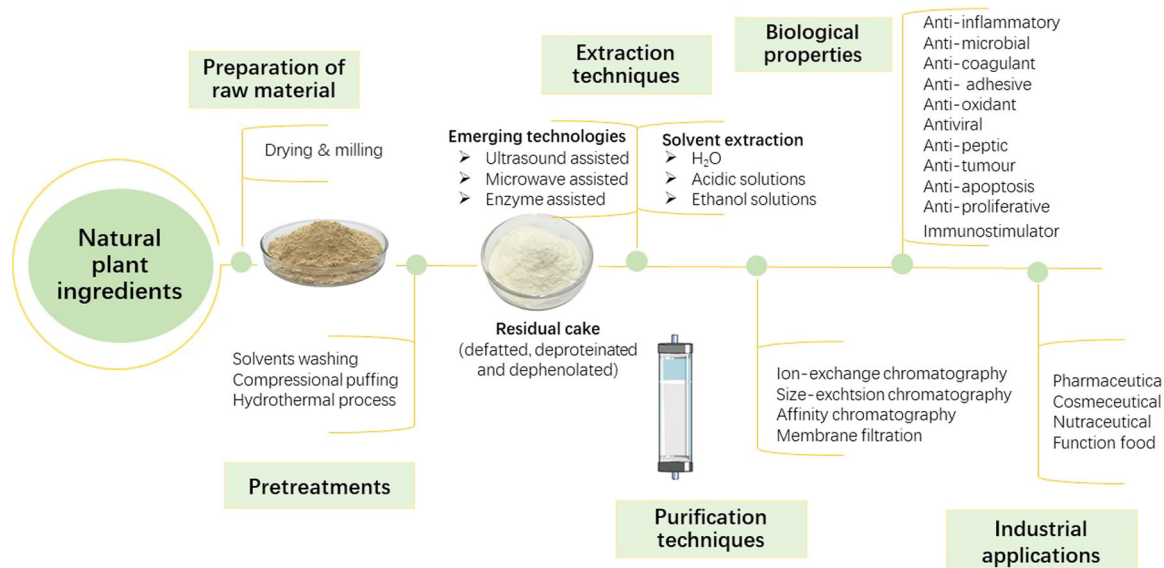


FIGURE 3 Processes, activities, and applications of various extraction methods for polysaccharides.

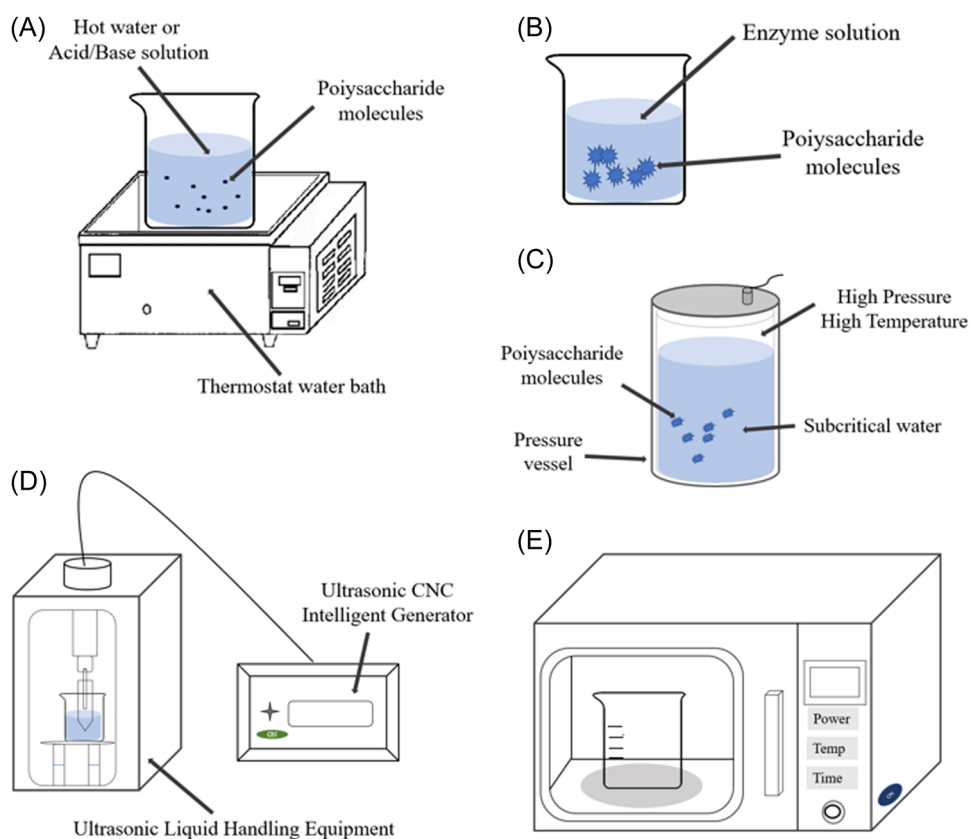


FIGURE 4 Polysaccharide extraction method: (A) Polysaccharide extraction by hot water or acid/base solution. (B) Polysaccharide extraction by enzyme. (C) Polysaccharide extraction by subcritical water. (D) Polysaccharide extraction by ultrasonic. (E) Polysaccharides extraction by microwave.

in polysaccharides with lower molecular weight and higher ratio of neutral sugars to uronic acid (X. Chen et al., 2020). The acid degradation rate of polysaccharides is generally positively correlated with the extraction temperature and

acidity value, and acid extraction is preferentially used to extract sulfated acidic polysaccharides. In the extraction of acidic marine polysaccharides, mild acidic conditions can transfer the hydrolysis of fucoidan from the 2-sulfated ester

to a specific site adjacent to the 4-sulfated unit, and then cleave the nonsulfated unit. The higher the temperature of the acid, the greater the degree of cleavage of polysaccharides, resulting in an increase in the proportion of sulfated fucose. Acid-induced cleavage of glycosidic bonds does not alter the primary structure of plant polysaccharides. Under alkaline conditions, the alkaline solution can easily cause swelling of the *Hericium erinaceus* cell wall by destroying the hydrogen bonds within polysaccharides, leading to the degradation of galactoglucomannans and improving the release of β -(1 $\rightarrow$ 3)-glucans (Yi et al., 2020). In addition, different concentrations of alkali during the extraction process also have effects on the physicochemical properties and microstructure of polysaccharides. Compared to HWE, the molecular weight and particle size of the three different concentrations of alkaline-extracted polysaccharides (MBP 0.5, MBP 1.0, and MBP 2.0) were reduced. Furthermore, at low alkali concentration, the content of galacturonic acid in polysaccharides is higher, and the molecular structure becomes smaller, showing smaller molecular weight and particle size. But at higher alkali concentration, some free polysaccharides will reassemble, resulting in more vital intermolecular forces, dense structure, flat surface, and stronger gel network structures (Chen, Qin, et al., 2022).

1.3 | EAE

Enzymatic extraction is a relatively mature and stable extraction method of polysaccharide extraction methods. It is usually used to release polysaccharides from cell walls and degrade macromolecular substances such as starch or protein under suitable environmental conditions, commonly used enzymes include neutral protease, cellulase, papain, and pectinase (Cheng et al., 2015; Duan et al., 2018). The structures of polysaccharides depend on the selected enzymatic hydrolysis method. Due to the high specificity and selectivity of hydrolase, compared to other extraction methods, enzymatic extraction affects mainly the composition of monosaccharides and the proportion of polysaccharides (Qin et al., 2022). Compared to acid/alkaline extraction, polygalacturonic acid (HG) hydrolase could enzymolysis the α -(1,4)-glycosidic bond between GalA residues in enzymatic extraction, thus lessening the structure of HG and the preparation of pectin polysaccharides rich in the RG-I domain under milder conditions. The results of monosaccharide analysis showed that polysaccharides extracted by the enzymatic method had the largest side chains of neutral sugars such as galactose and arabinose, and the higher degree of branching resulted in a higher molecular weight. Furthermore, compared to the HWE method, cellulase could eliminate the mannose of water-soluble polysaccharides in the mung bean peel (*Vigna radiate* Linn.), increase the proportion of fructose and reduce molecular weight (Jiang et al., 2020). α -amylase is often used in starchy food processing, but it can also affect

the structure of polysaccharides. For example, *Passiflora edulis* polysaccharides will be hydrolyzed by 25.3% after action, and the content of uronic acid will be reduced by approximately 50%. Moreover, mucinase also affects polysaccharide structure, such as the content of uronic acid, the degree of methyl esterification, and the molecular weight of *okra* polysaccharides will be decreased after the action of mucinase. For barley bran polysaccharides, three steps of enzymatic hydrolysis using α -amylase, protease, and glucoamylase (which can be replaced by pullulanase or xylanase) compared to the single action of α -amylase, significantly increased the β -glucan content of extract and decreased molecular weight (Yi et al., 2020).

1.4 | SWE

Subcritical water refers to hot liquid water at high temperature (100–374°C), and high pressure (5–10 MPa), the mixture of extraction raw materials and distilled water is added to the autoclave and the air is purged with inert gas before closing (J. Zhang et al., 2019). It is an environmentally friendly, fast, and efficient technology for the extraction of polysaccharides from plant materials. During the extraction of pectin, the proportion of glucose in polysaccharides gradually increased with processing temperature and time. When the SWE temperature was higher than 150°C for more than 15 min, high molecular weight components (>90, 000 Da) of polysaccharides were degraded. The triple-helix structure disappeared. When the extraction temperature is at medium and low temperature (100–140°C), a small amount of cellulose or hemicellulose can be extracted or degraded, resulting in higher molecular weight, high esterification degree, relatively high galacturonic acid content, and lower neutral sugar content of pectin polysaccharides, elevated temperature or prolonged extraction time accelerated the thermal degradation of the polymer, and the ionization constant also increased with increasing temperature, which enhanced the acidolysis of pectin polysaccharides GalA, Rha, and Ara, thereby increasing the acidity of subcritical water. However, Glc, Man, Xyl, and Gal were positively correlated with extraction temperature, so the pectin polysaccharides were severely degraded in high-temperature region (160–180°C), further increasing the content of neutral sugar and reducing the molecular weight (F. Zhang et al., 2022). Subcritical water can lead to differences in monosaccharide composition, change molecular structure, and affect the biological activity of polysaccharides (L. Wang et al., 2022).

1.5 | Ultrasonic-assisted extraction (UAE)

Ultrasound can generate intense cavitation, high shear, fragmentation, and stirring forces, thereby destroying the molecular chain and network structure of polysaccharides, but it will not change the primary chain connection,

monosaccharide composition, and glycosidic bond type of polysaccharides (H. Wang et al., 2021). In addition, ultrasound can easily lead to the cleavage of polygalacturonic acid chains of polysaccharides, the cleavage of intermolecular hydrogen bonds, and the increase in free carboxyl levels and galacturonic acid content (Wu et al., 2022). The molecular weight of polysaccharides decreased rapidly with an increase in ultrasonic power, frequency, and time. The maximum molecular weight of polysaccharides extracted by single-frequency, dual-frequency, and triple-frequency ultrasonic methods decreased with the superposition of single-frequency, and the composition of monosaccharides remained unchanged. However, the relative sugar content is different. For example, the most significant proportion of monosaccharides in polysaccharides treated by single-frequency, dual-frequency, and triple-frequency ultrasound were galactose, glucose, and arabinose (B. Yang, Luo, et al., 2020). Multifrequency sonication also effects on the surface stereo-shapes of polysaccharides (Figure 5), polysaccharides treated with triple-frequency ultrasound have pores that are closer than those treated with dual-frequency ultrasound, but both polysaccharides have amorphous structures (X. Chen et al., 2021). Single-frequency ultrasonic treatment can shorten the pectin molecular chain, but does not change the primary structure. During the ultrasonic treatment, the pectin chains of *Premna microphylla* Turcz (PEP) were rapidly fractured within the initial 10 min and then the degradation rate gradually slowed down. With the increase in ultrasonic time and intensity, the conformation of PEP pectin chains changed from

semiflexible chains to flexible chains, flexible coils, and even compact coils. *P. microphylla* Turcz pectin chains can be curled, folded, and tightened upon short-time, low-intensity sonication due to the disruption of steric hindrance and the increase of the terminal carboxyl group (-COOH) content. However, prolonged high-intensity sonication aggravated the fragmentation of the side chains of *P. microphylla* Turcz pectin and increased the number of short rigid chains. The self-curling of PEP pectin was inhibited due to the faster Brownian motion and stronger intermolecular hydrogen bonds of smaller molecules (Q. Shi et al., 2022).

1.6 | MAE

Microwave is an electromagnetic wave with a frequency of 300 MHz–300 GHz, and has functions such as heating, drying, and sterilization through molecular friction in the electromagnetic field (Y. Yang, Lei, et al., 2020). The polysaccharides degrade slightly, wrinkle, and fold at low microwave power. As frequency increases, the micro-structure and internal tissue are disrupted and ruptured, leading to easier solvent intrusion and increased release of high molecular weight polysaccharides (Oyeyinka et al., 2021). Furthermore, in some cases, side chains of different origins in polysaccharides are degraded during microwave irradiation, generate new functional groups, high molecular weight moieties are converted to low molecular weight, and change their initial structure, but not the primary structure of polysaccharides (X. Chen, Yang, et al., 2022). The ratio of neutral to

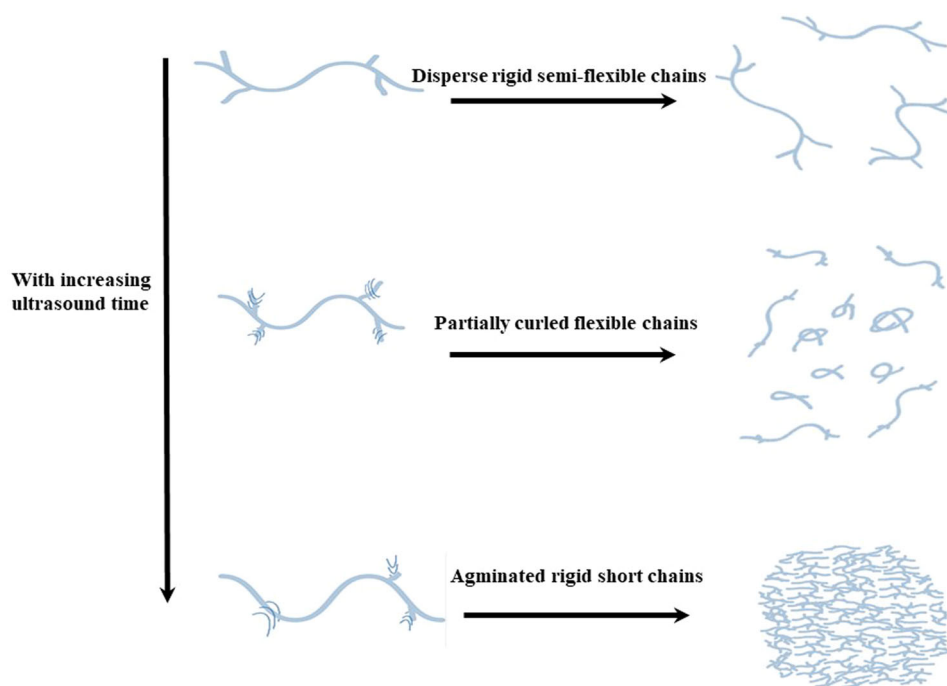


FIGURE 5 Polysaccharide conformation transition under sonication.

acidic polysaccharides components of microwave-treated flaxseed gum (FG) polysaccharides components was not significantly affected within 1–3 min. But as microwave treatment continued for 5 min, relatively higher proportions of arabinose, xylose, and glucose were found in FG polysaccharides, resulting in an increase in the proportion of neutral and acidic polysaccharide components.

At the same time, when the extraction temperature was increased from 30°C to 90°C, the proportion of neutral sugar and acidic sugar components of the FG polysaccharides continued to decrease, and the morphological structure of polysaccharides gradually changed to loose and curly (Yu et al., 2022). After the microwave power was increased to a high intensity of 650 W, the microwave radiation enhanced the cleavage rate of the glucosidic bond and resulted in a decrease in the number of hydroxyl groups. Compared to polysaccharides obtained by HWE, the content of sulfated, hydroxyl groups, and unmethylated uronic acid of polysaccharides extracted by MAE was higher, and the percentage of glucose was lower, better configuration changes favor hydrogen release from O–H bonds, also lead to smaller molecular size or lower molecular weight (Mirzadeh et al., 2020).

2 | CONCLUSION

Polysaccharides extracted by different methods, even if extracted from the same raw material, have considerable differences in structures and biological activities. This difference is closely related to structural features such as the molecular weight, type, and proportion of polysaccharides, and the position and configuration of glycosidic bonds. Factors such as high temperature, acid, alkali, ultrasound, microwave, and enzymes are the main reasons for the physical and chemical changes of natural polysaccharides during the preparation process. However, the structure–activity relationship between the structural characteristics of polysaccharides and various activities is a preliminary study, deepening analysis of the relationship between its structure and biological activity is the focus of future research on polysaccharides. Second, the commonly used extraction technology is simple and polysaccharide structure will be degraded during the extraction process, affecting its function. Therefore, understanding the degradation mechanism and exploring the effect of different novel extraction techniques (such as multimodal combined extraction) on polysaccharide structure. The establishment of an efficient and accurate research system through close integration with instruments, technologies, and methods is of great significance for the multiscale structural properties and morphological characteristics of polysaccharides under the action of free radicals, electric fields, and pressures, which in turn affect their functions and biological activities.

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DATA AVAILABILITY STATEMENT

Data sharing not applicable—no new data generated, or the article describes entirely theoretical research.

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