

In vitro Antioxidant Activities of Natural Polysaccharides: An overview

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Received: August 30, 2019

Accepted: September 24, 2019

Online Published: October 29, 2019

doi:10.5539/jfr.v8n6p78

URL: <https://doi.org/10.5539/jfr.v8n6p78>

Abstract

Polysaccharides are naturally occurring biomacromolecules composed of carbohydrate molecules linked by glycosidic bonds. A number of polysaccharides are known to possess beneficial therapeutic effects against inflammation, diabetes, cardiovascular diseases, and cancers. Indeed, polysaccharides are reportedly effective free radical scavengers and antioxidants, thereby playing a critical role in the prevention of damage to living organisms under oxidative stress. In this review we provide an overview of the sources, extraction, and antioxidant activities of some natural polysaccharides.

Keywords: polysaccharide, antioxidant activity, health benefits

1. Introduction

Polysaccharides are naturally occurring biomacromolecules composed of carbohydrate molecules linked by glycosidic bonds. These molecules play an important role in the development of pharmaceuticals, food, nutritional products, and biodegradable packaging materials (Yu, Shen, Song, & Xie, 2018; Copeland, Blazek, Salman, & Tang, 2009; Yang & Zhang, 2009; Cazon, Velazquez, Ramirez, & Vazquez, 2017). They are commonly derived from plants, fruits, vegetables, herbs, algae, mushrooms, and microorganisms (Kardasova & Machova, 2006; Zhang et al., 2019; Maity, Maity, & Dasgupta, 2019; Han et al., 2019; Hajji et al., 2019; Liu, Ooi, & Chang, 1997; Huang, Siu, Wang, Cheung, & Wu, 2013; Hu, Gen, Zhang, & Jiang, 2001; Guo et al., 2010; Kodali & Sen, 2008).

Polysaccharides can be either linear or branched and they can be classified as homopolymers if the polymer composed of identical monosaccharides, or heteropolymers, if the polysaccharide is composed of two or more different monosaccharides. The structural composition of polysaccharides is rather complex since the monosaccharide units linked through glycosidic linkages can be composed of different sequences, degrees of polymerization, and branching. As such, the structures of these compounds are determined using various sophisticated analytical and spectroscopic techniques, such as fourier transform infrared spectroscopy (FT-IR), ultraviolet spectrophotometry (UV-Vis), nuclear magnetic resonance (NMR) spectrophotometry, gas chromatography-mass spectrophotometry (GC-MS), and liquid chromatography-mass spectrophotometry (LC-MS). It has also been found that their structural properties vary based on the natural sources, processing methods, extraction methods, and variety of agricultural or botanical species (Wang, Hu, Nie, Yu, & Xie, 2016; Wang et al., 2013; Cui, 2005).

One of the major applications of polysaccharides is their uses as a staple food source for humans. For example, starch is key ingredient in various foods supplying 50–70% of the energy in a human diet. Upon digestion, starch can be hydrolyzed to glucose, which is an integral part of the development of brain and red blood cells. The rate of digestibility of polysaccharides has an active role in determining the glycemic index (GI), i.e., the level of glucose in the blood. More specifically, a rapid rate of digestibility results in a higher GI and can leads to complications such as type II diabetes and obesity. However, polysaccharides that exhibit beneficial health

properties are of great interest in the food industry as a number of these compounds have been shown to exhibit anti-inflammatory, antidiabetic, and anticancer properties, in addition to preventing cardiovascular diseases (Yu, et al., 2018; Copeland et al., 2009; Yang & Zhang, 2009; Cazon et al., 2017; Kardasova & Machova, 2006). Polysaccharides are also used in many biomedical and biological applications, such as tissue engineering, drug delivery, wound healing, and biosensors (Yu, Shen, Song, & Xie, 2018; Copeland, Blazek, Salman, & Tang, 2009; Kardasova & Machova, 2006; Zhanget al., 2019). More importantly, some have been employed as antioxidants both *in vitro* and *in vivo* due to their free radical scavenging properties that play a critical role in the prevention of damage from oxidative stress. Thus, in this review, we provide an overview of the sources, extraction, *in vitro* antioxidant properties of some natural polysaccharides.

2. Sources of Polysaccharide

2.1 Plant Polysaccharides

Among the various natural polysaccharides, the major polysaccharides tend to be extracted from plant materials. Several polysaccharides isolated from plant sources have been reported to exhibit significant antioxidant activities both *in vivo* and *in vitro*. For example, two polysaccharides isolated from *Rosa rugosa petals* (RRPS, namely RRPS-1 and RRPS-2) were analyzed for their antioxidant activities and it was found that RRPS-2 had a good radical scavenging potential (Zhang et al., 2019). In addition, a water soluble arabinoxylan (APPS), with a molecular weight of $\sim 1.49 \times 10^5$ Da was isolated from the green stem of *Andrographis paniculate* and showed strong antioxidant activity against superoxide and hydroxyl free radicals (Maity, Maity, & Dasgupta, 2019). Kiwifruit polysaccharides also exhibited high antioxidant activities (Han et al., 2019). Furthermore, Hajji et al., (2019) isolated a novel water-soluble polysaccharide (PLP1) from the root barks of *Periploca laevigata* by hot water extraction and further purification by diethylaminoethyl cellulose (DEAE)-sepharose chromatography, and this compound exhibited a strong antioxidant activity. Recently, Wei et al., (2019) isolated polysaccharides from seabuckthorn berries (PSB) and investigated the role of microwave power on their antioxidant activities. Furthermore, a water-soluble polysaccharide (MWP) with a galactose-, glucose-, mannose-, fructose with 1:33.2:8.4:7.2 was successfully isolated from *Malus micromalus* Makino fruit (Hui, Jun, & Chuang, 2019) and this polysaccharide showed degrees of scavenging effects on hydroxyl, DPPH (2,2-diphenyl-2-picrylhydrazyl), and superoxide radicals in a dose dependent manner. Zhu et al., (2009) isolated two major polysaccharide fractions composed of arabinose, galactose, and glucose, which were associated with protein portions from Huaza No. 4 rapeseed meal using column chromatography and ethanol precipitation, and this compound also exhibited a significant antioxidant activity (Zhu and Wu, 2009). In addition, three polysaccharides with molecular weights of 218, 178, and 60 kD were extracted from the roots of *Arctium lappa L.* (ALPs) and were reported to exhibit strong scavenging activities for DPPH, hydroxyl, and superoxide radicals both *in vitro* and *in vivo* (Jiang et al., 2019). Furthermore, Litchi pulp polysaccharide extracted from Litchi *Chinensis* Sonn. showed a dose dependent antioxidant activity against DPPH, hydroxyl, and superoxide radicals, (Kong et al., 2010) while Ginkgo biloba leaves (GBLs), a common herbal remedy, exhibited noticeable scavenging effects on superoxide radicals and ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radicals (Ren et al., 2019).

2.2 Fungal Polysaccharide

Fungal cell walls are composed of polysaccharides such as glucans, chitin, and mannans, which contain glucose, N-acetylglucosamine, and mannose repeating units, respectively. However, the structures of these species also differ; for example glucans are composed of glycosidic linkages including branched glucans and linear glucans, while chitin is a water insoluble polymer composed of β 1,4-linked N-acetylglucosamine units. Studies into the beneficial effects of these polysaccharides indicated that mushrooms polysaccharides tend to exhibit significant antioxidant properties. For example, *Inonotus oblique* mushrooms produce a crude extract polysaccharide, which is widely used in Russian folk medicine and is reported to have significant medicinal and antioxidant properties (Mu et al., 2012). In addition, a polysaccharide from *Hericium erinaceus* mushrooms exhibits a strong *in vitro* antioxidant activity in addition to neuroprotective effects on β -induced neurotoxicity in neurons (Cheng, Tsai, Lien, Lee, & Sheu, 2016). Furthermore, polysaccharides extracted from the edible mushroom *Tricholoma mongolicum* exhibited *in vitro* antioxidant activities in a dose-dependent manner (Zhao et al., 2016) while fucogalactomannan obtained from the *Tylophilus ballouii* mushroom inhibited superoxide and hydroxyl radicals with IC_{50} values of 1.25 and 1.6 mg/mL, respectively (Lima et al., 2016). Moreover, a water-soluble β -glucan isolated from the hot water extract of the edible mushroom *Entoloma lividoalbum* (Kühner & Romagn) Kubička stimulated the production of macrophages, splenocytes, and thymocytes, in addition to exhibiting hydroxyl and superoxide radical scavenging activities and reducing properties (Maity, et al., 2015). Indeed, it has been reported that antioxidant activities of these fungi originated from their component polysaccharides and

polysaccharide-protein complexes (Tseng, Yang, & Mau, 2008; Wu & Wang, 2009; Alzorqi, Sudheer, Lu, Manickam, 2016; Chen, Zhong, Zeng, & Ge, 2008). In this context, Chen et al., (2008) reported that the administration of polysaccharides lentinans from *Lentinus edodes* could decrease the oxidative stress induced by a high-fat diet in rats (Chen, Zhong, Zeng, & Ge, 2008).

2.3 Bacterial Polysaccharide

Bacterial polysaccharides are biomacromolecules that are anchored to the cell surface by lipid and exist as extracellular polysaccharides, peptidoglycans, lipopolysaccharides, capsules and exopolysaccharides. Bacterial stains such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Bordetella bronchiseptica*, *Actinobacillus pleuropneumoniae*, and *Yersinia pestis* contains high level of polysaccharides in the form of extracellular polysaccharides (EPS). More specifically, Guo et al., (2010) reported that two polysaccharide isolated from the marine bacterium *Edwardsiella tarda* exhibited a good antioxidant activity against hydroxyl and DPPH radicals, while Kodali et al., (2008) isolated an EPS from the probiotic bacterium *Bacillus coagulans* and confirmed that it exhibited a significant antioxidant activity (Guo et al., (2010); Kodali & Sen, 2008). Moreover, Raza et al., (2011) reported that an EPS from *Paenibacillus polymyxa* possessed a good superoxide scavenging activity in addition to a moderate inhibition of lipid peroxidation (Raza, Makeen, Wang, Xu, & Qirong, 2011).

2.4 Animal Polysaccharide

Animal polysaccharides as glycogens composed of α -1,4-glycosidic bonds with branched α -1,6 bonds present at approximately every tenth monomer. These polysaccharides are used to store energy and are produced by the liver and the muscles during glycogenesis. Some examples of polysaccharides found in the human body are hyaluronic acid, chondroitin sulfate, dermatan sulfate, keratan sulfate and heparin. A study into the antioxidant activity of a polysaccharide from *Hyriopsis cumingii* (HCP) showed both *in vitro* and *in vivo* free radical scavenging, in addition to an increase in the activities of antioxidant enzymes in a dose-dependent manner (Qiao et al., 2009). A free neutral polysaccharide was extracted from the mucus of the loach (*Misgurnus anguillicaudatus*) and their effects on the removal of reactive oxygen species were examined. Indeed, the efficiently removal of $\cdot\text{O}_2$, $\cdot\text{OH}$, H_2O_2 and other active oxygen compounds were confirmed and these polysaccharides were found to significantly protect DNA chains from damage by free radicals (Zhang, Wang, & Dong, 2011). Chitin is one of the main components of cell walls in fungi, radulas of mollusks and is a linear homopolysaccharide comprising N-acetyl-glucosamine. Ngo et al., (2008) employed acidic hydrolysis for the isolation of a polysaccharide from crab chitin and upon investigation of its cellular antioxidant effects, they observed free radical scavenging effects (Ngo, Kim, & Kim, 2008).

2.5 Algal Polysaccharide

Fucoidan is a natural sulfated polysaccharide that contains fucose and is known to exhibit numerous beneficial health properties due to the presence of the sulphate ester group. Algal polysaccharides are generally found in the form of alginates, fucans, laminarans, cellulose, and sulfated galactans. More specifically, Zhang et al., (2010) found that fucoidan had promising antioxidant activity toward superoxide radicals rather than hydroxyl and DPPH radicals and brown algae containing sulfated polysaccharides showed significant antioxidant properties (Zhang et al., 2010). In addition, Sokolova et al., (2011) reported that sulfated polysaccharides, namely carrageenans, isolated from red algae possessed strong antioxidant activities both *in vivo* and *in vitro* due to stimulation of the catalytic activity of superoxide dismutase (SOD) (Sokolova et al., 2011). Both the molecular mass and sulfate content in fucoidan play a significant role in determining its radical scavenging activity and, a positive correlation with the sulfate content has been demonstrated. It was also reported that the sulfated and acetylated derivatives of polysaccharides extracted from the red algae *Gracilariopsis lemaneiformis* exhibited strong antioxidant activities, although certain derivatives exhibited stronger activities than the unmodified compounds (Wang et al., 2019).

3. Polysaccharide Extraction, Isolation and Characterization

Water is commonly employed as a solvent for the extraction of polysaccharides. However hot water was found to be better extracting solvents than the cold water. Several polysaccharides were extracted from various sources as listed in the table 1 by using water with temperatures 50-100 °C. Higher temperature improved the extraction of polysaccharide. In most of the cases the polysaccharides have been isolated using chromatography such as anion chromatography, DEAE column chromatography and gel filtration after ethanol precipitation, and deproteination. Figure 1 represents a general strategic extraction and purification of polysaccharide from various polysaccharide sources. Pure Garlic polysaccharide was extracted by using hot water. These polysaccharides were isolated and separated by column chromatography on Sephadex G200 and washed with distilled water and then

characterised by ^{13}C and ^{31}P NMR spectroscopy (Chen & Huang, 2019). Pure pumpkin polysaccharides were extracted by hot water after purification by ethanol precipitation and dialysis. Further sulfated and phosphorylated pumpkin polysaccharides were characterized by IR and ^{13}C NMR spectroscopy (Chen & Huang, 2019). Cushaw polysaccharide have been extracted with water at $100\text{ }^{\circ}\text{C}$ after ethanol precipitation followed by dialysis and sulfated and phosphorylated polysaccharide have been prepared with different degrees of substitution. Substituted polysaccharides have been characterized by IR and NMR spectroscopy (Chen & Huang, 2018). Chen et al., (2019) extracted and isolated polysaccharide with potential antioxidant activity from native ginseng by hot water and characterized by IR and NMR spectroscopy (Chen & Hunag, 2019).

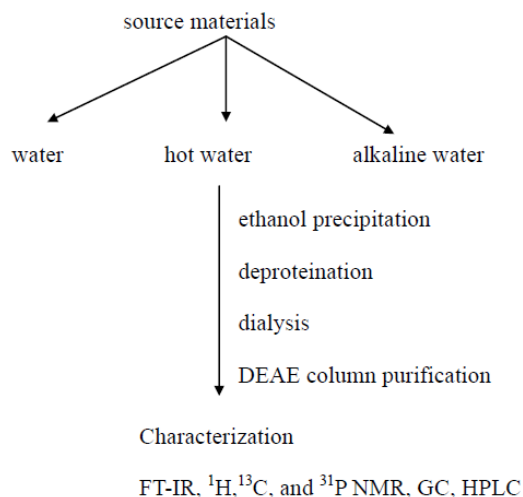


Figure 1. Schematic representation of extraction and characterization of polysaccharides

It has been observed that some advanced treatments such microwave irradiation, sonication, and other irradiation treatments have also been adopted to optimize polysaccharide extraction (Table 1). Furthermore, the extracted crude polysaccharides have been successfully purified by column chromatography.

Table 1. Sources, extraction methods, purifications, characterizations for some polysaccharides

sources	solvent	treatment and/or processing	characterisation	ref
Polygonatum sibiricum	water	microwave, ethanol precipitation, diethylaminoethyl cellulose-52 anion-exchange chromatography	high performance gel chromatography (HPGC)	Zhang et al., (2019)
Fritillaria pallidiflora	water	anion-exchange and gel-permeation chromatography	FT-IR, NMR, SEM	Rozi et al., (2019)
kiwifruit	water	microwave, ultrasound	FT-IR, high performance size exclusion chromatography (HPSEC), HPLC	Han et al., (2019)
Periploca laevigata	water	DEAE-sepharose chromatography	UHPLC, GC-MS, FT-IR, ¹ H NMR and ¹³ C NMR	Haaji et al., (2019)
Ginkgo biloba leaves	water	DEAE (diethylaminoethanol) Sepharose Fast Flow column	high performance liquid chromatography (HPLC), IR	Ren et al., (2019)
Inonotus obliquus	water	anion-exchange DEAE cellulose column and Sephadex G-200 gel	IR, Liquid Chromatography, HPGPC,	Hu et al., (2016)
Mung beans	alkaline water	purified and separated by anion exchange and gel filtration	HPSEC, GC	Yao et al., (2016)
Anji Baicha	hot water	-	-	Zhang et al., (2016)
Paecilomyces hepiali HN1	hot water	-	HPLC	Wu et al., (2016)
Poria cocos	hot water	ultrasonic, microwave, enzyme assisted	IR	Wang et al., (2016)
Artemisia sphaerocephala	water	ultrasound assisted, sulfated with triphenylchloromethane	HPLC, IR	Zheng et al., (2016)
Tangerines peel	water	microwave assisted, purified by anion exchange and gel filtration	IR	Chen et al., (2016)
Blackcurrant fruit	water	pressurised water extraction	GC, HPLC, and IR	Xu et al., (2016)
Dipsacus asperoides	water	ultrasonic power		Tan et al., (2015)
Chrysanthemum morifolium	hot water	anion exchange, DEAE -cellulose column, gel filtration	partial acid hydrolysis and NMR	Zheng et al., (2015)
Rana chensinesis	water		HPLC, GC, IR	Wang et al., (2015)
Stachys sieboldii Miq	water	DEAE Sepharose, Sepharose CL-6B	HPLC, GC	Feng et al., (2015)
Fortunella margarita	water	ultrasonic power, purified DEAE Sepharose, Sepharose CL-6B	UV-Vis, HPLC, NMR	Zeng et al., (2015)
Rana chensinesis	water	DEAE Sepharose, Sepharose CL-6B	-	Wang et al., (2015)
Radix Cyathulae officinalis	water	Water soluble	IR, GCMS, GPC	Han et al., (2015)
Cucurbita moschata	water	ultrasound and enzyme	-	Wu et al., (2014)
Isochrysis galbana	water	anion exchange chromatography and gel	IR, ESI-MS, NMR	Sun et al., (2014)
Tremella fuciformis	water	Fe ²⁺ /Vc/H ₂ O ₂	UV-Vis, IR, HP-GC	Zhang et al., (2014)
garlic	petroleum ether, acetone, methanol	cellulase assisted	-	Pan et al., (2014)
Ficus microcarpa	water	ultrasound	IR spectroscopy	Jiang et al., (2014)

4. Mechanisms of Antioxidant Properties of the Polysaccharide

4.1 Antioxidant Activities by Crude Polysaccharide Extract and Polysaccharide Conjugates

Numerous literatures exist regarding the health benefits of polysaccharides, where most of the studies were carried out using crude polysaccharide extracts. Interestingly, the presence of proteins, amino acid, peptides, organic acid, and other components appeared to enhance the antioxidant properties since, the purified polysaccharides tended to exhibit significantly lower antioxidant activities than the crude polysaccharides. For example, Ennaifer et al., (2018) reported an antioxidant-rich decoction *Pelargonium graveolens* containing a water-soluble polysaccharide (Ennaifer, Bouzaine, Chouaibi, & Hamdi, 2018). Also, Zhang et al., (2019) isolated three polysaccharide extract with and without proteins and found that the protein-containing polysaccharide extract exhibited the highest antioxidant activity (Zhang et al., 2019). In another study, Huang et al., (2003) reported that polysaccharides isolated from *Cordyceps sinensis* showed no antioxidant activity following separation of the protein from the crude (Huang, Liang, Guo, Zhou, & Cheng, 2003). In addition, Siu et al., (2016) investigated the antioxidant activities of crude polysaccharide from mushrooms (*L. edodes*, *G. frondosa*, and *Zt. Versicolor*) and found that their antioxidant activities were positively correlated with the contents of phenolic acids and proteins (Siu, Xu, Chen, & Wu, 2016). Indeed, the formation of polysaccharide complexes with other components such as amino acids, proteins, and lipids are particularly common, yielding polysaccharide conjugates. Interestingly, majority of reports indicate that the polysaccharide conjugates exhibit higher antioxidant activities than the free polysaccharides or bioactive compounds. It has been suggested that polysaccharide-polyphenols and polysaccharide-protein conjugates are formed via hydrophobic interactions and hydrogen bonding. Recently Barmudez et al., (2019) reported that interactions between polysaccharides and phenolic compounds contributed to a strong free radical scavenging activity through a combination of covalent (ester bonds) and non-covalent (hydrogen bonding and electrostatic interactions) interactions (Barmuez et al., 2019). It was found that the interactions after drying were stronger than those found in the simple mixture. For example, phenolic acids (secondary metabolites in many plant species) form polysaccharide conjugates that exhibit significant antioxidant activities. Indeed, Wang et al., (2012) investigated the antioxidant activities of oolong tea and found no antioxidant activities from the phenolic acid-free and protein-free extracts (Wang, Yang, & Wei, 2012).

Polyphenol associated polysaccharides are also known to contribute significantly to the antioxidant activity determined by using the DPPH assay. In some cases, polyphenol-free tea polysaccharides have also been found to exhibit a strong antioxidant activity. Thus, to examine the role of polysaccharide polyphenol complexes in determining the antioxidant activity of such species, Wu et al., (2011) carried out a comparative study using tea polyphenols, β -glucan, and the corresponding mixture and complex, and found that the complex exhibited the highest antioxidant activity against hydroxyl free radicals (Wu et al., 2011). However, inconsistent results were found for the mixture, thereby demonstrating that a complex structure of tea polyphenols and β -glucan plays a critical role in determining the antioxidant activity. Ferulic acid polysaccharide complexes have also been found to exhibit strong antioxidant activities. Moreover, Shu et al., (2019) investigated the role of particle size in green tea powder and found that the antioxidant activity could be improved by reducing the particle size (Shu, Li, Yang, Dong, Wang, 2019).

Selenium is a biologically relevant element found in soil and agricultural products. However, organoselenium compounds have been reported to exhibit more beneficial properties than free selenium species. To mimic the enhanced bioavailability and biological properties of organoselenium compounds, a series of selenium compounds have been synthesized using biologically relevant molecules such as proteins, amino acids, alkaloids and polysaccharides. For example, Wei et al. (2012) reported that a selenylated polysaccharide (Se- RHP) synthesized from *Radix hedysari* exhibited an enhanced antioxidant activity compared to the free polysaccharide (Wei, Cheng, Wei, & Zhang, 2012). Similar polysaccharide selenium complexes have been synthesized by Wang et al., (2012) using a polysaccharide extracted from *Artemisia sphaerocephala* (Wang, Zhao, Wang, Yao, & Zhao, 2012). In addition, in the case of *Potentilla anserina* L. Zhao et al., (2013) successfully improved the antioxidant activity compared to the native polysaccharides through the formation of a complex with selenium (Zhao et al., 2013). The higher antioxidant activity of the polysaccharide was attributed to conformational changes in the polymers containing a higher number of hydroxyl groups. Furthermore, Yu et al., (2007) reported that Se-enriched green tea had a higher antioxidant activity than the regular tea due to the formation of polysaccharide-Se complexes, while polysaccharides isolated from Se-enriched *G. lucidum* were more effective in reducing the production of superoxide radicals (Yu , Sheng, Xu, An, & Hu, 2007; Zhao et al., 2008). Moreover, Mao et al., (2014) and Cheng et al., (2013) studied the polysaccharide isolated from *G. frondosa* and found that the Se-enriched polysaccharide (Se-GP) was a stronger scavenger for DPPH, ABTS, and hydroxyl

radicals (Mao et al., 2014; Cheng et al., 2013). However, no significant differences were observed between these species in terms of the polysaccharide content and the molecular weight, with the Se content being the only variable. Interestingly, Guo et al., (2013) reported that a selenium-polysaccharide synthesized by using selenium chloride oxide (SeCl_2O) and the polysaccharide also exhibited a higher total antioxidant capacity, superoxide radical scavenging effect, and hydroxyl radical scavenging effect (Guo et al., 2013).

4.2 Antioxidant Properties of Processed Polysaccharide

The biological properties of polysaccharide are dependent on their structural composition, conformation, and type of glycosidic linkage. Moreover, the sources, extraction, and processing methods, significantly influence the polysaccharide structure and conformation, which can subsequently alter the chemical and biological properties. Interestingly, number of reports have indicated a correlation between the molecular weight and radical scavenging activity of polysaccharides (Liu, Wang, Pang, Yao, & Gao, 2010; Xing et al., 2005; Zha et al., (2009); Sun, Wang, Shi, & Ma, 2009; Zhang, Wang, Mo, & Qi, 2013, Choi et al., 2009). It was hypothesized that polysaccharides with low molecular weights would possess a greater number of reductive hydroxyl group terminals to accept and eliminate free radicals, since the number of hydroxyl or amino groups in polysaccharide molecules such as chitosan greatly influence the free radical scavenging activity. For example, Zhang et al., (2019) isolated four polysaccharide fractions (P-1: 71.40%, P-2: 1.95%, P-3: 1.14%, and P-4: 1.64%) from crude *Polygonatum sibiricum* polysaccharide (PSP), and the antioxidant capacity was P-4 > P-3 > P-2 > PSP > P-1 (Zhang et al., 2019). In addition, two low molecular weight polysaccharides were isolated from *G. lucidum* by Liu et al., (2010) and these were reported to display significant antioxidant activities (Liu, Wang, Pang, Yao, & Gao, 2010). Similarly, Xing et al., (2005) observed higher scavenging activities by polysaccharides with lower molecular weights, which was partly attributed to differences in their structural composition compared with higher molecular weight species (Xing et al., 2005). Furthermore, Zha et al.(2009) obtained three polysaccharide fractions from rice bran with molecular weights ranging from 1.2×10^5 to 6.3×10^6 Da (PW1), 3.5×10^4 to 7.4×10^4 Da (PW2), and 5.3×10^3 to 2.3×10^4 Da (PW3), and they found that PW3 exhibited the best reducing power, chelating metal ion potential, and scavenging ability against DPPH and ABTS radicals (Zha et al., 2009).

To improve the taste and quality of raw materials for foodstuffs, processing is commonly carried out using a range of methods, including cooking. During such processing, polysaccharides are transformed or degraded; for example, treatment with γ -rays enhances the antioxidant activity as the polysaccharide structure is retained, but fragmentation to lower molecular weight polysaccharides took place (Choi et al., 2009). Treatment with ultrasonic waves also degrades the polysaccharide structure and enhances the antioxidant activity (Zhou, Wang, Ma, & He, 2008). In addition, various chemical treatment methods have been adopted for the processing of foods, including the use of ascorbic acid and hydrogen peroxide to degrade the polysaccharide obtained from *Enteromorpha linza*. (Zhang, Wang, Zhao, & Qi, 2014). However, contrasting results were obtained. More specifically, although Sun et al., (2009) reported that high molecular weight polysaccharides exhibited no significant antioxidant activities, and low molecular weight polysaccharides displayed strong antioxidant activities (Sun, Wang, Shi, & Ma, 2009). Cheng et al., (2013) found that higher molecular weight polysaccharides from *Epimedium acuminatum* exhibited higher antioxidant activities than the lower molecular weight polysaccharides (Cheng et al., 2013). In contrast, some other studies have suggested that the effect of molecular weight was not significant based on the similar antioxidant levels of polysaccharides and oligosaccharides (Kardosova & Machov, 2006).

4.3 Antioxidant Activities of Functionalized Polysaccharides and Polysaccharides Bearing Different Functional Groups

The structural characteristics of a polysaccharide play a significant role in determining its potential antioxidant activity. Although polysaccharides bearing only an oxygen heteroatom do not exhibit significant antioxidant properties derivatization through sulfation, carboxylation, phosphorylation, benzylation, acetylation, or oxidation produces promising antioxidants. For example, sulfated polysaccharides are major derivatives that exhibit significant antioxidant activities and protect against lipid peroxidation. Yang et al., (2011) confirmed that the sulfated polysaccharides from *Corallina officinalis* displayed a higher antioxidant potential than the desulfated species (Yang, Liu, Wu, & Chen, 2011). A similar observation was reported by Hu et al., (2010) for the polysaccharides extracted from *Undaria pinnatifida*, while Wang et al., ((2009) reported that native sulfated polysaccharides isolated from *Laminaria japonica* showed a significant antioxidant activity (Hu, Liu, Chen, Wu, 2010; Wang et al., 2009). Indeed, it has been reported that the antioxidant capacity of a sulfated polysaccharide depends on the degree of substitution on the polysaccharide backbone, since the degree of substitution has a critical impact on polymer conformational and structural changes. In addition, Qi et al., (2015) isolated four different polysaccharides with varied molecular weight and reported that low molecular weight polysaccharides

had a higher reducing power (Qi et al., 2005). Furthermore, Zhang et al., (2011) reported a strong antioxidant activity for the sulfated polysaccharide isolated from fresh persimmon (*Diospyros kaki* L.) fruit, while Wu et al., (2013) reported the development of acetylated, phosphorylated, and benzoyleated levan-type exopolysaccharide from *Paenibacillus polymyxa* EJS-3. Moreover, Wei et al., (2012) modified phosphorylated polysaccharides from *Radix hedysari*, while Qi et al., (2006) acetylated and benzoyleated derivatives from the polysaccharides of *Ulva pertusa*. (Qi et al., 2006; Wei, Cheng, Wei, & Zhang, 2012).

Pectic acid, chitosans and alginates are some other form of polysaccharides that exhibit high antioxidant activities. However due to the cationic or anionic nature of these polysaccharides, the presence of sulfated or phosphorylated glucans resulted in different antioxidant potentials (Qi et al., 2005). For example, Chen et al. (2009) reported that agar bearing a sulfate group, and chitosan bearing an amino group exhibited antioxidant activities, while starch bearing a hydroxyl group did not (Chen, Tsai, Huang & Chen, 2009). In a similar study Rao and Muralikrishna observed that glucose and soluble starch, laminarin, did not show any antioxidant activity (Rao & Muralikrishna, 2006). In addition, acidic polysaccharides containing uronic acid displayed a higher antioxidant activity due to the presence of electrophilic keto or aldehyde groups (Chen, Zhang, & Xie, 2004). Interestingly, uronic acid polysaccharides exhibit a different levels of antioxidant properties, decreasing in the order polygalacturonic acid > glucuronic acid > galacturonic acid. This difference was attributed to the nature of the carbonyl group (i.e., chain or ring). Furthermore, Li et al. (2011) reported that polysaccharides isolated from *Zizyphus Jujuba* cv. *Jinsixiaozao* exhibited different antioxidant activities due to the presence of varying levels of uronic acid with polysaccharide fractions containing higher uronic acid contents giving stronger free radical scavenging activities than fractions containing no uronic acid (Li, Liu, Fan, Ai, & Shan, 2011).

Fucoidan is a natural sulfated polysaccharide found in algae and it has been shown to have number of health benefits. Wang et al. (2009) extracted fucoidan from *Laminaria japonica* and derivatized it to its sulfated, phosphorylated, benzoyleated and aminated forms, all of which exhibited strong antioxidant activities (Wang et al., 2009). In addition, Feng et al., (2010) modified the lentinan polysaccharide found in mushrooms to give its sulfated derivative, which again exhibited a significant antioxidant activity (Feng, Li, Wu, He, & Ma, 2010). Compared to the native polysaccharide extracted from *Portulaca oleracea* L. Chen et al., (2014) reported that phosphorylated polysaccharides produced higher antioxidant activities (Chen et al., 2014). In addition, Wang et al., (2014) phosphorylated the galactomannan polysaccharide and found that a higher degree of phosphorylation correlated positively with the antioxidant activity (Wang et al., 2014). Furthermore, Jung et al., (2011) reported higher antioxidant activity upon increasing the degree of sulfation, while Wang et al., (2013) found that a higher degree of sulfation in the polysaccharide isolated from the green algae *Enteromorpha linza* gave a higher DPPH scavenging activity (Jung, Bae, Lee, & Lee, 2011; Wang et al., 2013). In contrast, Xie et al., (2015) found that sulfated polysaccharides with the highest degree of substitution did not produce the highest antioxidant activities.

Based on these results for functionalized polysaccharides it was hypothesized that substitution on a polysaccharide chain weakens the dissociation energy of the hydrogen bonds, and so the hydrogen donating ability of the polysaccharide derivatives is greater than that of the unmodified species. Alternatively, abstraction from the anomeric carbon may be promoted. However, chemical modification is often accompanied by a decrease in molecular weight, thereby improving the antioxidant potentials of the polysaccharides. In terms of sulfated polysaccharides, free radicals are often trapped in an electrostatic manner since the sulfate groups tend to generate a highly acidic environment, while substitution by sulfur may also weaken the hydrogen bonding between polysaccharides (Wang et al., 2016). Furthermore, the types of substitution and the degree of substitution appeared to influence the physicochemical properties (e.g. molecular weight, polarity, solubility, and charge density) and conformation of native polysaccharides. The degree of substitution may also affect the activity through interruption of the inter and intramolecular hydrogen bonds (Wang et al., 2016). It is also possible that saccharides (carbohydrates) can become antioxidants in alkaline media at pH values >9. In such a medium, an amperometric sensor can directly detect saccharides without the requirement for derivatization, thereby selecting only the target antioxidants (Wang, Zhang, Yao, Zhao, & Qi, 2013). Moreover, in an alkaline medium, dissociation occurs through the removal of hydrogen from the hydroxyl groups of saccharides. This does not take place in other media (acid and/or neutral), and so in such cases, the amperometric sensor does not detect the presence of any saccharides.

Antioxidant activities of polysaccharide have been associated with diverse forms of polysaccharide including polysaccharide conjugate, polysaccharide complex, functionalized polysaccharide and degraded products of polysaccharide (Figure 2). In addition to the significant antioxidant properties of polysaccharides, these macromolecular systems also exhibit anticancer and antiviral properties, immune regulation and other biological activities. More specifically, the anti-cancer properties of polysaccharides have been reported to occur through

either the induction of cellular apoptosis and cell cycle arrest, or by inhibiting tumor invasion, adhesion and metastasis. In addition, synergistic effects have been reported in combination treatment with conventional anti-cancer drugs (Chen & Huang, 2018; Lovegrove et al., 2017). Polysaccharides are known to play a significant role in human health, since the rate of digestion, adsorption, and metabolism of polysaccharides is regulated in the stomach, small intestine, and large intestine thereby controlling colonic health and vascular function. Dietary polysaccharides therefore have the potential to regulate the intestinal microenvironment and stimulate macrophages or lymphocytes in the gut tissues to prevent cancers (Huang, Nie, & Xie, 2017; Laurienzo, 2010). Dietary fiber and resistant forms of starch therefore play a positive role in reducing risk factors for chronic diseases, including cardiovascular disease and certain types of cancers.

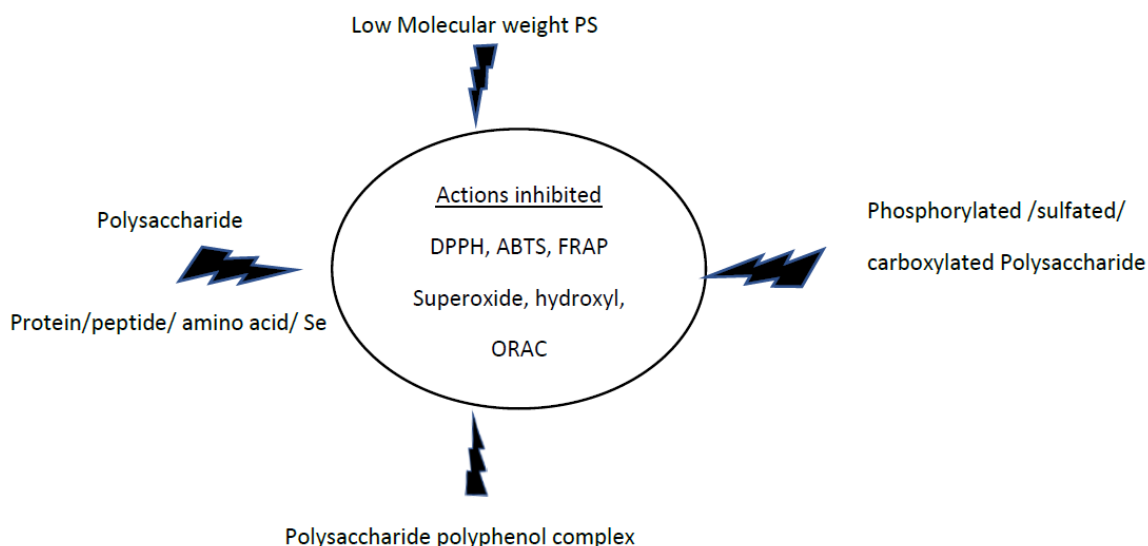


Figure 2. Polysaccharide species for antioxidant activities

5. Conclusion

Natural antioxidants exhibiting strong antioxidant activities are commonly employed to protect against oxidative damage, yet little is known regarding the antioxidant effects of polysaccharides. Although polysaccharide-rich foodstuffs assigned a high glycemic index (i.e. rapidly digested to give glucose) suspected to have links with obesity, diabetes, and high blood pressure, recent findings into the physiological effects of polysaccharides, polysaccharide conjugates, and polysaccharide complexes with biorelevant molecules results in slower glucose release and provides physiological health benefits. Therefore, modified polysaccharide and slowly digested polysaccharides are the most desirable types of polysaccharides from a nutritional standpoint. We expect that recent findings into polysaccharides exhibiting antioxidant properties may lead to a better understanding of the potential of natural polysaccharides to act as functional antioxidants, due to their high antioxidant activities. This area is therefore in need of further exploration in future studies.

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