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The antioxidant activity of polysaccharides: A structure-function relationship overview

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ABSTRACT

Over the last years, polysaccharides have been linked to antioxidant effects using both *in vitro* chemical and biological models. The reported structures, claimed to act as antioxidants, comprise chitosan, pectic polysaccharides, glucans, mannoproteins, alginates, fucoidans, and many others of all type of biological sources. The structural features linked to the antioxidant action include the polysaccharide charge, molecular weight, and the occurrence of non-carbohydrate substituents. The establishment of structure/function relationships can be, however, biased by secondary phenomena that tailor polysaccharides behavior in antioxidant systems. In this sense, this review confronts some basic concepts of polysaccharides chemistry with the current claim of carbohydrates as antioxidants. It critically discusses how the fine structure and properties of polysaccharides can define polysaccharides as antioxidants. Polysaccharides antioxidant action is highly dependent on their solubility, sugar ring structure, molecular weight, occurrence of positive or negatively charged groups, protein moieties and covalently linked phenolic compounds. However, the occurrence of phenolic compounds and protein as contaminants leads to misleading results in methodologies often used for screening and characterization purposes, as well as *in vivo* models. Despite falling in the concept of antioxidants, the role of polysaccharides must be well defined according with the matrices where they are involved.

1. Introduction

Polysaccharides are energy storage and/or support and act as signaling components of the cell walls of plant, fungi, bacteria, and algae. In animals, polysaccharides are found as extracellular matrix cell components, as well as part of their exoskeletons (Lovegrove et al., 2017). They are relevant food components as energy source, as is the case of starch, or even modulate the organoleptic properties of food, especially the mouthfeel as food texture modulator (Yang, Li, Li, Sun, & Guo, 2020) as well as astringency (Soares, Brandão, Mateus, & de Freitas, 2017).

Industrials and informed consumers are aware of the relevance of polysaccharides on Human nutrition, as they have been related to several health benefits, mainly as dietary fibre (Lovegrove et al., 2017) and cholesterol binding capacity of the different structures, including fructans (Espinosa-Andrews, Urías-Silvas, & Morales-Hernández, 2021) and β-glucans (Mejía, de Francisco, & Bohrer, 2020). According to Scopus database, assessed on December 2022, by performing a search for "polysaccharide" and "antioxidant", >10,000 works also relate or attribute antioxidant features to polysaccharides. This assignment is supported by the capacity of polysaccharide to scavenge *in vitro* chemically generated radicals as hydroxyl radical ($OH^{\bullet}$), superoxide ($O_2^{\bullet -}$), 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\bullet +}$), 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), among others. Some studies also claim polysaccharides as antioxidants accounting *in vitro* effects on the antioxidant, anti-inflammatory, immune-modulatory, metabolism and proliferation in distinct cell lines (Lahrsen, Liewert, & Alban, 2018; Schneider, Ehrig, Liewert, & Alban, 2015; Siu, Chen, & Wu, 2014). Despite this huge amount of data, the fact that for most polysaccharides there are no clear structure/function relationships, still raises several questions. Are polysaccharides relevant antioxidants? What is the impact of food processing on polysaccharides antioxidant activity? Is there contaminant effects by antioxidants such as phenolic compounds, vitamins (E and C) and carotenoids? Can the isolation of polysaccharides promote changes in their antioxidant activity? These are some of the questions that this review target to answer, providing an overview of the structural features and mechanisms that might confer antioxidant properties to the different polysaccharide structures isolated from plant,

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animal, algae, fungi, yeast, and bacteria. Aiming to cover as many polysaccharide structures as possible, the references cited were selected based on their contribution to disclose the structure/function relationships related to polysaccharides antioxidant activity. This included all studies using purified and well characterized polysaccharides whose antioxidant activity has been measured.

2. Common methods used to measure polysaccharides antioxidant activity

2.1. Hydroxyl radical ($OH^{\bullet}$)

The $OH^{\bullet}$ can be generated from $O_2^{\bullet-}$ and H_2O_2 (Fig. 1). This radical is one of the most reactive radical species. As a result, all compounds can promote a scavenging activity towards $OH^{\bullet}$, acting as antioxidants. This limits any extrapolation of the results obtained with this radical to possible *in vivo* antioxidant effects. However, considering that $OH^{\bullet}$ results from $O_2^{\bullet-}$ and H_2O_2 as precursors, as well as through iron or copper ions chelation, experiments using this radical are useful to disclose mechanisms of antioxidant action (Gulcin, 2020; Magalhães, Segundo, Reis, & Lima, 2008).

For polysaccharides (Table 1), the $OH^{\bullet}$ scavenging can be measured accounting the presence of free Fe^{3+} in the presence of H_2O_2 in neutral buffered solutions (Ueno et al., 2012). This strategy allows to consider both the $OH^{\bullet}$ scavenging and the iron chelation as antioxidant mechanisms of polysaccharides. This approach can be followed by electron spin resonance (ESR) spectrometry (Park, Je, & Kim, 2004), as well as by spectrophotometric methods. For polysaccharides, the most common is based on a Fe^{2+}+H_2O_2 + salicylic acid system. This methodology is based on $OH^{\bullet}$ generation, assured by the reduction of Fe^{3+} to Fe^{2+} by an OH-salicylic acid adduct, and on the hydroxylation of salicylic acid, measured at 510 nm (Hu et al., 2016; Ning et al., 2021; Wang et al., 2019). Alternatively, as used for many other antioxidants, 2-deoxy-D-ribose can be used as target on Fe^{3+}+H_2O_2 + ascorbic acid system. In this case, the 2-deoxy-D-ribose degradation products are determined by heating with thiobarbituric acid solutions at acidic pH, yielding a pink chromogen that can be measured spectrophotometrically at 532 nm (Magalhães et al., 2008). These results are frequently expressed as EC_{50} concentration values (Table 1), *i.e.*, the concentration of polysaccharide required to decrease the radical concentration in 50 %.

The represented polysaccharide structural and antioxidant features, although representative of the different polysaccharides with potential antioxidant activity, do not provide an exhaustive and detailed information regarding their source, structural features and modifications. "N. d" means "not determined" and "-"represents no scavenging ability of the tested polysaccharide.

2.2. Superoxide anion radical ($O_2^{\bullet-}$)

The superoxide anion radical ($O_2^{\bullet-}$) is formed by an electron transfer to molecular dioxygen (O_2). Despite being a weak oxidant, $O_2^{\bullet-}$ forms the highly oxidizing hydroxyl radicals ($OH^{\bullet}$) by reaction with H_2O_2 (Gulcin, 2020). As it is a common product of several cell metabolic processes (Halliwell, 2006), $O_2^{\bullet-}$ radical scavenging is frequently explored to assess polysaccharides antioxidant activity (Table 1), allowing to predict possible *in vivo* effects (Gulcin, 2020; Magalhães et al., 2008).

Among the available methods for $O_2^{\bullet-}$ scavenging, the most relevant ones rely on radical generation by using of xanthine oxidase with hypoxanthine or xanthine as substrates, approaching this model to biological systems. The generated $O_2^{\bullet-}$ is measured following its oxidation to O_2 by an oxidizing probe, usually nitroblue tetrazolium (NBT) that is converted into formazan. This reaction changes the color of the solution from the yellow of NTB to the blue of formazan, allowing its measurement spectrophotometrically at 560 nm (Rocha de Souza et al., 2007; Ueno et al., 2012). In the presence of antioxidants, the $O_2^{\bullet-}$ is reduced to H_2O_2, leading to a lower formation of formazan (Gulcin, 2020; Magalhães et al., 2008). Chemiluminescent methods using luminol analogous (Isaka et al., 2015) and ESR spectrometry (Park et al., 2004) can also be used for the determination of $O_2^{\bullet-}$ scavenging activity.

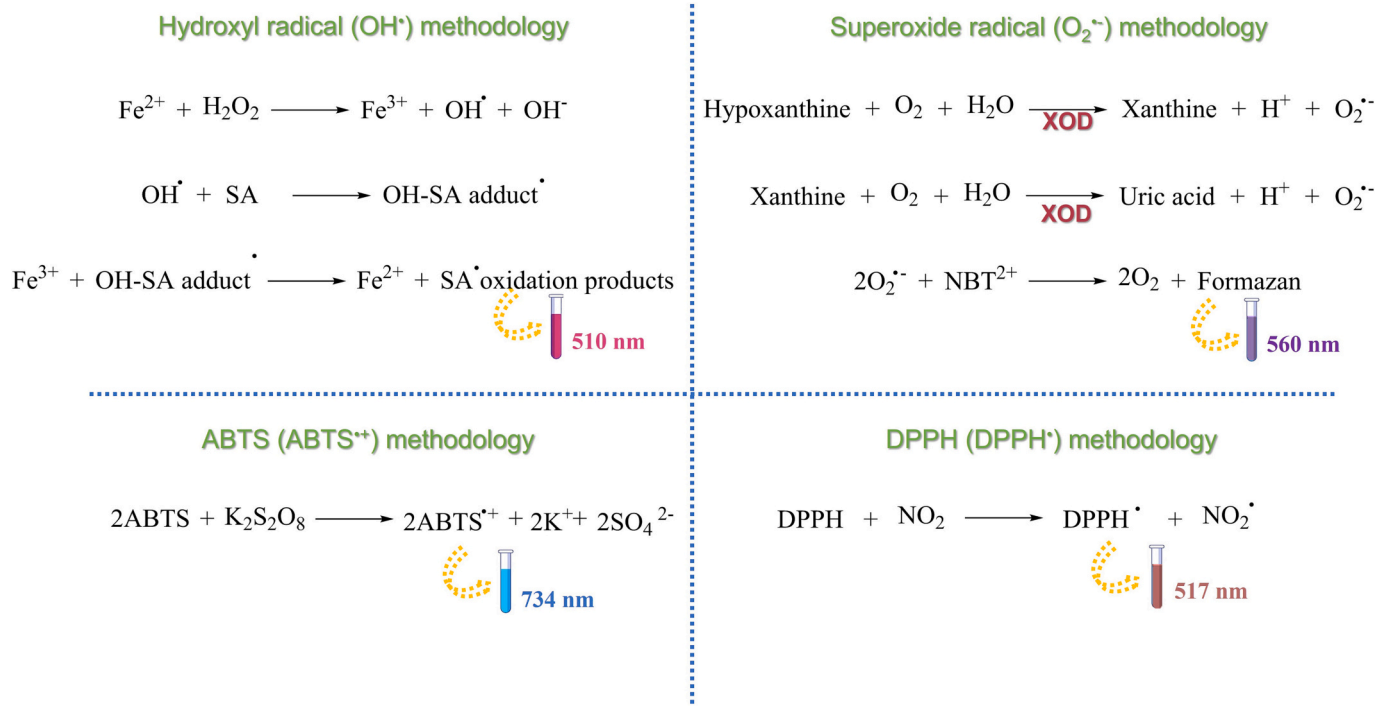


Fig. 1. Most popular antioxidant methods employed for the determination of polysaccharides antioxidant activity. SA – Salicylic acid; XOD – Xanthine oxidase; NBT - Nitrotetrazolium Blue chloride.

Table 1
Polysaccharides enriched fractions with reported antioxidant properties in terms of their effective concentration to reduce the radical concentration in 50 %, EC_{50} values (mg/mL).

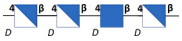
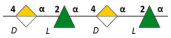
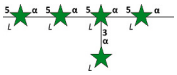
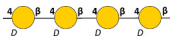
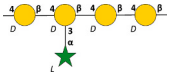
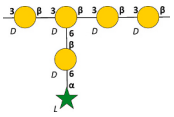
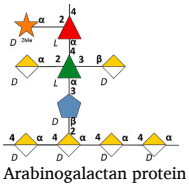
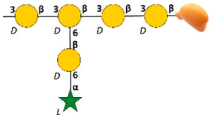
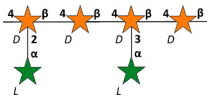
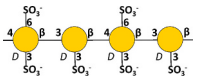
Polysaccharide		$OH^{\bullet}$	$O_2^{\bullet-}$	$ABTS^{\bullet+}$	$DPPH^{\bullet}$	Reference
Positively charged polysaccharides	Chitosan	0.4 to >2	5.5 to >35	0.21 to >24	0.87 to >10	(Sun, Zhou, Xie, & Mao, 2007; Tomida et al., 2009; Yang, Guo, Miao, Xue, & Qin, 2010)
						
	Carboxymethylated derivative	>2	>1	n.d.	≥3	(Bai, Yong, Zhang, Liu, & Liu, 2020; Liu, Lu, Kan, Tang, & Jin, 2013; Yang et al., 2010)
	Phenolic grafted derivative	0.5 to 0.8	0.3 to 1	0.2	0.3 to 0.6	(Liu et al., 2013; Wang et al., 2019)
Negatively charged polysaccharides	Homogalacturonan	4 to 5	0.7 to 0.9	2–3	>5	(Karaki, Aljawish, Muniglia, Humeau, & Jasniewski, 2016; Ning et al., 2021; Wang & Lü, 2014)
						
	Phenolic grafted	n.d	n.d	11	1	(Karaki et al., 2016)
	Type I Rhamnogalacturonan	147 to 153	5–8	n.d.	17 to 22	(Ning et al., 2021)
						
	Arabinan-rich pectic polysaccharides	4–19	4	n.d.	3 to 20	(Golbargi, Gharibzahedi, Zoghi, Mohammadi, & Hashemifesharaki, 2021; Ning et al., 2021)
						
	Galactan-rich pectic polysaccharides	8	2	n.d.	6	(Ning et al., 2021)
						
	Type I Arabinogalactans	2 to 3	>10	2	≥4	(Li et al., 2023; Petera et al., 2015; Wang et al., 2018)
						
	Type II Arabinogalactans	2	n.d	1–3	2–3	(Ahmadi, Rezadoost, Alilou, Stuppner, & Moridi Farimani, 2022; He et al., 2018)
						
	Rhamnogalacturonan (RG-II)-rich pectic polysaccharides	7–9	1–2	n.d.	9–15	(Ning et al., 2021)
						
	Arabinogalactan protein	0.2 to 3	0.3	n.d.	12	(Aguirre, Isaacs, Matsuhiro, Mendoza, & Zúñiga, 2009; Yamassaki, Campestrini, Zawadzki-Baggio, & Maurer, 2018)
						
	Xylan	>2	>2		>2	(Hu et al., 2016)
						
	Arabinoxylans	0.2 to >2	0.5 to >2	3	3 to 10	(Hromádková, Paulsen, Polovka, Košťálová, & Ebringerová, 2013; Hu et al., 2016; Maity et al., 2019; Xiao et al., 2022)
						
	Phenolic grafted	n.d.	n.d.	0.5 to 1	3 to >5	(Xiao et al., 2022)
	λ-Carragenan	0.4	0.05	n.d.	n.d.	(Rocha de Souza et al., 2007)
						

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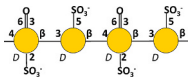
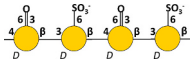
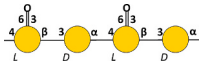
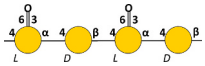
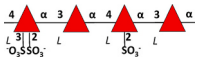
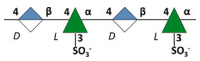
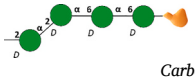
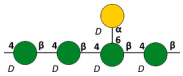
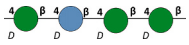
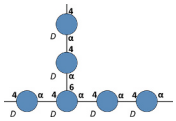
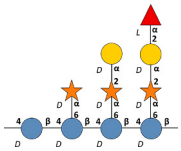
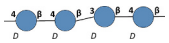
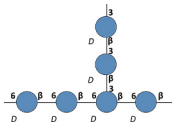
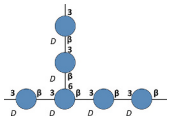
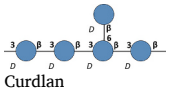
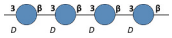
Table 1 (*continued*)

Polysaccharide		$OH^{\bullet}$	$O_2^{\bullet-}$	$ABTS^{\bullet+}$	$DPPH^{\bullet}$	Reference
	ι-Carragenan	0.3	0.3	n.d	n.d	(Rocha de Souza et al., 2007)
						
	κ-Carragenan	0.3	0.1	n.d.	n.d.	(Rocha de Souza et al., 2007)
						
	Porphyran	0.07	0.2 to 0.7	n.d	n.d	(Isaka et al., 2015; Zhou, Yu, Zhang, He, & Ma, 2012)
						
	Agarose	2	n.d	n.d	2	(Souza et al., 2012)
						
	Alginate	0.4 to 2	0.1	n.d.	0.2 to 0.6	(Hentati et al., 2018; Sellimi et al., 2015; Zhao, Li, Xue, & Sun, 2012)
						
	Fucoidan	0.2 to >4	0.03 to 0.2	n.d.	0.4 to >4	(Hentati et al., 2018; Rocha de Souza et al., 2007; Wang et al., 2009)
						
	Sulfated derivative	2.6	0.008	n.d.	>4	(Wang et al., 2009)
	Benzoylated derivative	0.5	0.007	n.d.	>4	(Wang et al., 2009)
	Acetylated derivative	2.1	0.008	n.d.	>4	(Wang et al., 2009)
	Ulvan	4	0.009 to 0.02	n.d	n.d	(Qi et al., 2005; Qi et al., 2006)
						
	Benzoylated derivative	2	0.02	n.d	n.d	(Qi et al., 2006)
	Acetylated derivative	0.9	0.01	n.d	n.d	(Qi et al., 2006)
	Mannoprotein (yeast)	3	n.d.	n.d.	>4	(Galinari, Almeida-Lima, Macedo, Mantovani, & Rocha, 2018; Liu & Huang, 2018; Machová & Bystrický, 2013)
						
	Carboxymethylated	2	2.5	n.d	n.d	(Liu & Huang, 2018)
	Phosphorylated	2	3.2	n.d	n.d	(Liu & Huang, 2018)
Neutral polysaccharides	Galactomannan	0.8 to >2	≥2	n.d.	5	(Hu et al., 2016; Wang et al., 2014)
						
	Phosphorylated	1 to 4	1	n.d.	1 to 2	(Wang, Yang, et al., 2014)
	Glucomannan	>2	>2	>2	>2	(Hu et al., 2016)
						
	Starch	>2	n.d	–	–	(Wen, Ye, Zhu, & Zhao, 2016; Yang et al., 2010)
						
	Carboxymethylated	>2	n.d	n.d	n.d	(Yang et al., 2010)
	Phenolic grafted	n.d	n.d	n.d	0.4–1.9	(Wen et al., 2016)
	Xyloglucans	>2	>0.4	n.d	>0.5	(Cao & Ikeda, 2009)
						
	Selenious ester derivative	0.7	0.1	n.d	0.2	(Cao & Ikeda, 2009)
	Sulfated derivative	2	0.4	n.d	>0.5	(Cao & Ikeda, 2009)

(*continued on next page*)

Table 1 (*continued*)

Polysaccharide		$OH^{\bullet}$	$O_2^{\bullet-}$	$ABTS^{\bullet+}$	$DPPH^{\bullet}$	Reference
	3,4 β-Glucan	>10	>10	n.d.	100	(Qian, Bai, Tang, & Chen, 2015; Shah, Gani, Masoodi, Wani, & Ashwar, 2017)
	 *Sulfated version*	2 to 8	6 to 10	n.d.	4 to 10	(Qian et al., 2015)
	3,6 β-Glucan	0.3 to 0.4	0.08 to 0.1	3 to 4	4 to 5	(Ashraf Khan, Gani, Masoodi, Mushtaq, & Silotry Naik, 2017; Maity et al., 2015; Nandi et al., 2014)
	 3 β-Glucan	–	n.d.	n.d.	–	(Machová & Bystrický, 2013; Tang et al., 2017)
	 *Carboxymethylated*	2	3	n.d.	>1	(Machová & Bystrický, 2013)
	Sulfated	>3	>3	n.d.	n.d.	(Tang et al., 2017)
	Phosphorilated	1	2	n.d	n.d.	(Tang et al., 2017)
	Laminarans	8	4	>3.	5	(Giese et al., 2015; Sellimi et al., 2018)
	 Curdlan	>3	n.d	>3	>2	(Giese et al., 2015; Wang, Zhang, Qiao, Cai, & Yan, 2021)
	 *Phenolic grafted*	n.d	n.d	n.d	1	(Wang et al., 2021)
Reference antioxidants	Gallic acid	0.06	0.1	n.d	0.009	(Fernandes et al., 2019; Yamassaki, Campestrini, Zawadzki-Baggio, & Maurer, 2017)
	Quercetin	n.d	0.03	n.d.	0.009	
	Ascorbic acid	0.007	0.1	0.003	0.003	
	Butylated hydroxytoluene (BHT)	n.d	n.d.	n.d.	0.02	

2.3. 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) method

$ABTS^{\bullet+}$ is a cationic radical produced by the oxidation of ABTS reagent. Potassium persulfate is usually used as oxidant, forming an intense blue color (Gulcin, 2020; Magalhães et al., 2008). The solubility of ABTS reagent in water, as well as the solubility of polysaccharides, allows the use of this method for the determination of polysaccharides antioxidant activity. The color decay is measured spectrophotometrically at 734 nm (Ahmadi et al., 2022; Ning et al., 2021; Wang et al., 2021; Xiao et al., 2022), attributed to the reduction of $ABTS^{\bullet+}$ following hydrogen atom and electron transfer mechanisms (Fig. 1).

The simplicity behind the $ABTS^{\bullet+}$ turns it one of the most used methods when screening polysaccharides antioxidant activity. Unlike many other antioxidant methods, data representation includes, beside EC_{50} values (Table 1), equivalents of standard water-soluble compounds as Trolox and ascorbic acid, among others (Gulcin, 2020; Magalhães et al., 2008). The use of different standards is a limiting factor for the comparison of polysaccharides antioxidant activity. As $ABTS^{\bullet+}$ is a synthetic radical, extrapolation to biological effects is not possible, only allowing comparison between the polysaccharide structures.

2.4. 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) method

$DPPH^{\bullet}$ is a frequent method used for a first screen of the antioxidant activity because of the high stability of the nitrogen radical species, attributed to the electron delocalization along the molecule (Gulcin, 2020; Magalhães et al., 2008). This radical is only soluble in organic solvents, usually ethanol, preventing a generalized application to measure polysaccharides antioxidant activity that tend to be insoluble in these solvents. Nevertheless, given its simplicity, low cost, and fast results, it is still applied for polysaccharides and oligosaccharides that can be solubilized in organic media (Table 1).

The $DPPH^{\bullet}$ solution presents a violet color measured at 517 nm. When reacting with an antioxidant, the color fades, attributed to the reduction of the radical to diphenyl-2-picryl hydrazine *via* hydrogen atom transfer mechanisms (Gulcin, 2020). Given that $DPPH^{\bullet}$, as $ABTS^{\bullet+}$, is a non-physiological radical, it can only be considered to screen the antioxidant capacity of organic solvent soluble polysaccharides, commonly expressed as EC_{50} values (Table 1). Besides this spectrophotometric approach, ESR spectrometry can also be used (Park et al., 2004).

3. Chitosan as an antioxidant polysaccharide

3.1. Chitosan characteristics and antioxidant activity

Chitosan is a positively charged polysaccharide composed of D-GlcN units linked by β1 → 4 linkages. As it is obtained by partial deacetylation of chitin, 10–60 % of D-GlcN units might still present acetyl amino groups at C-2 in the form of D-GlcNAc residues (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). While chitin is insoluble in water, the amino group of chitosan, with a pKa of 6.2 to 6.5, confers solubility depending on the pH of the solution. Chitin chains can form stable intra and intermolecular hydrogen bridges between the O and H atoms, as well as hydrophobic interactions governed by the C—H groups, including those present in the acetyl function, responsible for its insolubility (Sannan, Kurita, & Iwakura, 1976). At neutral and alkali conditions, the unprotonated chitosan amino groups are also involved in these interactions, explaining the chitosan insolubility (Lu, Song, Cao, Chen, & Yao, 2004). On the contrary, at acidic pH, the protonated amine groups confer positive charges to the polysaccharide, promoting repulsion of the chains, allowing that water molecules solvate the polymer, rendering them soluble. The higher the number of protonated amino

groups, the higher the solubility of chitosan (Lu et al., 2004; Nilsen-Nygaard, Strand, Vårum, Draget, & Nordgård, 2015). Chitosan average molecular weight commonly ranges from 9 to 700 kDa (Kou, Peters, & Mucalo, 2022).

Chitosan is reported to present radical scavenging activity, including $OH^{\bullet}$, $O_2^{\bullet -}$, $DPPH^{\bullet}$, and $ABTS^{\bullet +}$ radicals (Table 1). The scavenging of these radicals is negatively correlated with the degree of acetylation (DA) of chitosan due to the stabilization of oxidized structures provide by the amino group (Park et al., 2004; Tomida et al., 2009). In this sense, chitin, as a highly acetylated polysaccharide, present a very low radical scavenging capacity when compared with chitosan.

3.2. Antioxidant activity of chitooligosaccharides

Pools of chitooligosaccharides with a molecular weight ranging from 2 to 15 kDa, derived from chitosan partial depolymerization, are reported to present radical scavenging in experiments performed at pH 10.2 for $O_2^{\bullet -}$ and at pH 7.4 for $OH^{\bullet}$. The lower the molecular weight, the higher their antioxidant activity was reported (Sun et al., 2007; Tomida et al., 2009).

Despite oligosaccharides present a higher reducing end/extension unit ratio than polysaccharides, the carbonyl group of sugars only act as a reducing agent at high temperatures and alkali conditions as those found in the Fehling reaction where Cu^{2+} is reduced to Cu^{+} and precipitates in the form of Cu_2O (Fehling, 1849; Hall, 2003). Thus, the higher antioxidant activity of chitooligosaccharides is attributed to higher availability of amino groups provided by the open chain conformation at the reducing end of the chitooligosaccharides in opposition to the lower content of reducing ends of high molecular weight polymers (Nunes et al., 2016; Rocha, Coimbra, & Nunes, 2017). The decrease of the antioxidant activity of the chitooligosaccharides with the increase of the molecular weight can also be explained by the formation of intra and intermolecular interactions that decrease the solubility of the polymers (Kumar et al., 2004) and, consequently, the number of available amino groups (Park et al., 2004). Nevertheless, despite its insolubility, chitosan also exhibits antioxidant properties at alkali pH, attributed to the capacity of the amino group to donate a pair of electrons (Ngah, Ab Ghani, & Kamari, 2005), favoring Cu^{2+} (Chinnici, Natali, & Riponi, 2014) and Fe^{2+} (Nunes et al., 2016) chelation, involved on the formation of $OH^{\bullet}$ radicals *via* Fenton reactions.

3.3. Antioxidant activity of chitosan by interaction with other compounds

Due to the positive charges provided by NH_3^+ groups in acidic pH, chitosan can electrostatically interact with α-hydroxypolycarboxylic acids such as citric, malic, and tartaric acids. Due to their low pKa, they are present in aqueous solutions in unprotonated forms, allowing to have chelating properties and, simultaneously, interact electrostatically with chitosan, contributing to the antioxidant properties attributed to chitosan by entrapping oxidant metal ions (Rocha, Ferreira, Coimbra, & Nunes, 2020).

The positively charged chitosan is able also to interact with negatively charged phenolic compounds such as hydroxycinnamic acids (Rocha et al., 2017). These and other phenolic compounds can also be adsorbed to chitosan polysaccharide backbone by means of hydrophobic interactions and hydrogen bonding (Gassara et al., 2015; Popa, Aelenei, Popa, & Andrei, 2000). This phenomenon ascribes high reducing capacity to chitosan extracts as phenolic compounds, which are polyhydroxylated aromatic structures with unpaired electrons, when oxidized, yield highly stable radicals by resonance effects (Rice-Evans, Miller, & Paganga, 1996; Zeb, 2020). Thus, in these cases the antioxidant activity cannot be ascribed to the polysaccharide, but instead to the phenolic compounds that are retained to the polymeric chain that acts as a carrier.

The blend of positive charged chitosan with negatively charged pectic polysaccharides has been also proposed to confer higher antioxidant properties to materials for biomedical applications (Dziadek et al., 2022). To explain the contribution of negatively charged polysaccharides for the antioxidant activity, in the following section the antioxidant properties of these polysaccharides and their relationship with their structural features will be discussed.

4. Negatively charged polysaccharides

4.1. Pectic polysaccharides and alginate characteristics and their relation with antioxidant activity

Pectate (Pristov, Mitrović, & Spasojević, 2011) and alginate (Zhao et al., 2012) present $OH^{\bullet}$ radical scavenging in Fenton systems with metal/H_2O_2. The low pKa (3.3–3.8) of uronic acids, GalA in pectate and pectin (the methylesterified polysaccharide) as part of →4)-D-Gal*p*A-(α1 → chains of homogalacturonan domains (McNeil, Darvill, Fry, & Albersheim, 1984), and of D-ManA and L-GulA, found in alginate as →4)-D-Man*p*A(→β1 and →4)-L-Gul*p*A(→α1 units (Cao, Lu, Mata, Nishinari, & Fang, 2020; Draget & Taylor, 2011) suggest metal chelation as an antioxidant mechanism of these polysaccharides. This is supported by the retention of Ca^{2+}, a divalent ion like Fe^{2+} or Cu^{2+}, by the polysaccharides carboxylic and hydroxyl groups made available as an egg-box-like trap (Cao et al., 2020). In this way, these metal ions are unavailable to participate on $OH^{\bullet}$ generation. The fact that poly-D-mannuronate and poly-L-guluronate present close copper binding selectivity when in the presence of Cu^{2+}/Ca^{2+} solutions (Haug et al., 1970), together with the similar $OH^{\bullet}$ scavenging in iron Fenton system (Falkeborg et al., 2014; Ueno et al., 2012), is indicative that the metal chelation-based antioxidant activity of alginates is independent of its D-ManA and L-GulA proportion. In pectin, metal chelation is negatively correlated with its degree of methylesterification (DME) (Kyomugasho et al., 2017), attributed to the locking effect provided by the ester linkage. In this sense, in antioxidant systems where metal chelation is of relevance, pectin, as a highly methylesterified polysaccharide (>50 % DME), presents a lower antioxidant activity when compared with pectate (<50 % DME). Pectates, concerning copper chelation, are better antioxidants than alginates given their higher binding capacity when compared to poly-D-mannuronate and poly-L-guluronate (Haug et al., 1970). The antioxidant activity through metal chelation of xylogalacturonnans, Type I rhamnogalacturonnans and their arabinan, galactan and arabinogalactan side chains of pectic polysaccharides is expected to be low due to lower relative proportion of GalA in these polysaccharides.

4.2. Characteristics of uronic acid containing polysaccharides and their antioxidant activity

Xylans, usually defined as neutrally charged polysaccharides, contain, at the reducing end a →4)-D-Gal*p*A-(α1→ unit (Peña et al., 2007), which might also provide chelating properties. At a higher extent, glucuronoxylans and glucuronoarabinoxylans, highly substituted with D-Glc*p*A(α1→ and/or 4-*O*-Me-D-Glc*p*A(α1→ units (Ebringerová & Heinze, 2000; Sun & Sun, 2002) may also present metal chelating properties. Antioxidant activity provided by the chelating properties of uronic acids may also be expected from other polysaccharides of marine origin, as →4)-D-Glc*p*A-(β1→, →4)-L-Ido*p*A-(α1→, D-Glc*p*A(β1→ and L-Ido*p*A(α1→ of ulvans (Lahaye & Robic, 2007; Tziveleka, Ioannou, & Roussis, 2019) and →4)-D-Glc*p*A-(β1→ of fucoidan (Cong et al., 2016). Similarly, animal origin polysaccharides containing uronic acids, as →4)-D-Glc*p*A(β1→3)-D-Glc*p*NAc(β1→ units of hyaluronic acid (Tzianabos, 2000) may also contribute to the antioxidant properties of polysaccharides.

Besides metal chelation, negatively charged polysaccharides might establish interactions with metal ions forming coordination complexes that allow the direct scavenging of the $OH^{\bullet}$ generated adjacently to the polysaccharide backbone (Pristov et al., 2011). As the OH groups of

polysaccharides do not have reducing properties, $OH^{\bullet}$ scavenging is attributed to the high oxidative power of this radical (Niki, 2010). In the case of uronic acids, $OH^{\bullet}$ selectively abstracts hydrogen from the C-5 of the sugar, attributed to the steric and stereoelectronic effects provided by the proximity of the electron withdrawing carboxylic group (Gilbert, King, & Thomas, 1984; Hawkins & Davies, 1996). At pH above 4, this radical attack leads to sugar ring opening by β-scission reactions resulting on polysaccharide degradation by cleavage of the glycosidic linkages (Hawkins & Davies, 1996). The electronegative character of sulfate groups, if present in the C-2 position, is also reported to favor hydrogen abstraction by the anomeric carbon, providing antioxidant features to sulfated polysaccharides (Leal et al., 2018).

4.3. Characteristics of sulfated polysaccharides and their antioxidant activity

Negatively charged ester linked-sulfate groups can also contribute to polysaccharides antioxidant activity in Fenton systems (Campo, Kawano, Silva, & Carvalho, 2009; Lee et al., 2017; Murano, 1995; Tziveleka et al., 2019; Wang et al., 2009). As the sulfur atom is in its highest oxidation state, no reducing power can be attributed to sulfate groups (Leal et al., 2018). Instead, the available acidic moiety of the ester linked diprotic sulfate group forms ionic complexes with metals, allowing Fe^{2+} or Cu^{2+} chelation as reported for uronic acids, but with 10-fold less effectiveness (Haug et al., 1970). However, the antioxidant activity of heavily sulfated polysaccharides should be considered (Qi et al., 2005; Sun et al., 2015; Wang et al., 2009; Wang, Jiang, Mou, & Guan, 2004). Thus, accounting the sulfation patterns of algae polysaccharides, λ-carrageenan that present the highest average degree of sulfation, up to 40 %, distributed along the C-2, C-4 and C-6 of →3)-D-Gal*p*-(β1→ and C-2 of →4)-3,6An-D-Gal*p*-(α1→ units, also present the highest antioxidant activity, followed by ι-carrageenan and κ-carrageenan with 33 % and 20 % of degree of sulfation, respectively (Campo et al., 2009; Rocha de Souza et al., 2007). As fucoidans, sulfated at C-2 and C-4 of the →3)-L-Fuc*p*-(α1→ and 4)-L-Fuc*p*-(α1→ unit (Zhang, Zhang, Tang, & Mao, 2020), and of ulvans, sulfated at the C-3, C-2 or in both carbons of →4-L-Rha*p*-(α1→ and →2,4-L-Rha*p*-(α1→ units (Tziveleka et al., 2019), present a comparable average degree of sulfation, 23 %, they are prone to present equivalent antioxidant properties. Meanwhile, agar, which have a lowest average degree of sulfation, 16 %, present in at the C-6 of →4)-L-Gal*p*-(α1→ units as a precursor of →4)-3,6An-L-Gal*p*-(α1→ (Lee et al., 2017), and at C-4 and C-6 of →3)-D-Gal*p*-(β1→ units (Murano, 1995), should account with a lowest antioxidant activity in Fenton systems.

4.4. Characteristics of organic acids and phosphate substituted polysaccharides and their relation with antioxidant activity

Piruvate is also found in the galactose unit of polysaccharides as agar (Murano, 1995) and carrageenans (Chiovitti et al., 1997) and in cell wall and other polysaccharides of bacterial (Geissner, Pereira, Leddermann, Anish, & Seeberger, 2016) and yeast origin (Gemmill & Trimble, 1996) as a ketal linked group. In this context, the carboxylic acid moiety is available for metal chelation contributing to the antioxidant properties of pyruvate substituted polysaccharides. Other organic acids as succinate (Andreishcheva, Kunkel, Gemmill, & Trimble, 2004) and ether linked lactate (Lindberg, 1990) with available carboxylic groups can be part of many other polysaccharide structures (Andreishcheva et al., 2004), conferring antioxidant properties. In yeasts, as *Sacharomyces cerevisiae*, the →6)-D-Man*p*-(α1→, →2)-D-Man*p*-(α1→ and →3)-D-Man*p*-(α1→ units of mannoproteins might be present as mannosylphosphate, providing an acidic nature (Bastos et al., 2022; Jigami & Odani, 1999) that allows Fe^{2+} and Cu^{2+} chelation (Galinari et al., 2018), contributing to the antioxidant properties of mannoproteins. However, if Fe^{2+} is found as counter ion in acidic polysaccharides, as reported for fucoidans, the polysaccharide chelation properties are mitigated, promoting pro-oxidant activity (Abu et al., 2013). In this sense, it becomes of relevance to assure the removal of this element when assigning antioxidant features to polysaccharides. It can be done by ion competition with Ca^{2+} (Kurczewska, 2022) and/or chelating agents, as for instance EDTA.

4.5. Contribution of covalently bonded phenolics to polysaccharides antioxidant activity

Negatively charged polysaccharides also reduce other radical species, as is the case of $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals that, although not biologically relevant, are routinely used for *in vitro* antioxidant activity assessments (Fernandes, Ferreira, et al., 2019). However, as the sugar hydrogen atoms are not reactive towards these radicals, reported to have higher stability than the $OH^{\bullet}$ (Balaban et al., 1962; Guedes, Amaro, Gião, & Malcata, 2013), $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ reduction is usually used to evaluate phenolic compounds and protein antioxidant activity (Hu et al., 2016). When these are associated with polysaccharides, the essays using $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ do not allow to accurately determine the antioxidant activity conferred by polysaccharides. Besides, the majority of polysaccharides are not soluble in the hydrophobic solutions required to solubilize the DPPH reagent and evaluate antioxidant activities. This applies to ethanol, used as solvent for DPPH and for precipitation of polysaccharides. When this solvent is used, polysaccharides tend to form turbid solutions that, if not centrifuged, interfere with the results obtained by this colorimetric method.

As polysaccharides can present covalently attached phenolic compounds by means of biosynthetic processes, besides $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging, these polysaccharides also acquire metal reducing properties (Nimse & Pal, 2015), capacity to inhibit oxidative enzymes (Hromádková et al., 2013) and improved metal chelating properties given the contribution of the electronegative character of the polyhydroxylated phenolic aromatic ring. This is the case of pectic arabinans, galactan and arabinogalactans ester-linked to ferulic and coumaric acids derivatives in spinach (Fry, 1983) and sugar beet (Levigne et al., 2004; Rombouts & Thibault, 1986) or to arabinoxylans in bamboo (Ishii, 1991), and arabinoxylans and glucuronoarabinoxylans in cereals (Bunzel, Ralph, Marita, Hatfield, & Steinhart, 2001) and grasses (Hatfield, Rancour, & Marita, 2017). These antioxidant properties increase with the degree of polysaccharides substitution with phenolic compounds (Hromádková et al., 2013), usually in the Ara and Gal residues, at a sugar/phenolic compound proportion that might reach 1:1 for ferulic acid, and 15:1 for *p*-coumaric acid (Hatfield et al., 2017).

The polysaccharides covalent linkage to phenolic compounds might also occur from coupled oxidation reactions (Ferreira et al., 2002; Le Bourvellec, Guyot, & Renard, 2009), triggered by the enzymatic oxidation of phenolic compounds by polyphenol oxidase and peroxidases during plant tissue disruption (Guyot, Bernillon, Poupard, & Renard, 2008). It is possible that the occurrence of the linkages that have been evidenced in glucuronoxylans and pectic polysaccharides from olives (Coimbra, Waldron, & Selvendran, 1995), in pectic polysaccharides from cauliflower (Femenia, Rigby, Selvendran, & Waldron, 1999), in soluble pectic fractions of wine (Saura-Calixto & Díaz-Rubio, 2007), in pectic polysaccharides and arabinoxylans of apple (Stevens & Selvendran, 1984), in cell wall polysaccharides of pear (Ferreira et al., 2002) and even in algae fucoidans (Hentati et al., 2018) might have the contribution of phenolic compounds that react with polysaccharides. Oxidized phenolic compounds covalently linked to polysaccharides are also abundantly found in fruit pomaces (Fernandes et al., 2019). However, they can also be found as an artifact of sample manipulation for polysaccharide extraction (Renard, Lomax, & Boon, 1992) under alkali treatments (Hurrell & Finot, 1984). The type of linkages formed between the carbohydrate and phenolic moieties is unknown, but they are likely related to the aryl, diaryl-ether, peroxide, alkyl, aryl-ether and alkyl-aryl linkages of oxidized polyphenols (Fernandes et al., 2019; Fernandes, Le Bourvellec, et al., 2019). Polyphenol oxidation products also react with amino acids (Cao, Zhang, Zhang, Zhong, & Qian, 2009; Ishii et al., 2011) suggesting that, besides the sugar backbone, the protein moiety of type II

arabinogalactans might also be substituted with oxidized phenolic compounds. Given the occurrence of oxidation reactions during alcoholic beverages ageing (Fernández de Simón et al., 2014), covalently linked oxidized phenolic structures might also contribute to the antioxidant properties of yeast mannoproteins isolated from these foods and wine lees. However, as oxidized phenolics have lower reducing properties than the pristine compounds (Wong-Paz, Muñiz-Márquez, Aguilar, Sotin, & Guyot, 2015), these antioxidant polysaccharides are likely weaker antioxidants than those presenting equimolar substitutions with phenolic compounds *via* biosynthetic pathways.

Pear pinking during canning (Le Bourvellec et al., 2013) also shows that, if extracted at high temperatures and under acidic conditions, polysaccharides might also react with phenolic compounds, yielding covalent adducts with antioxidant properties. If reaching temperatures higher than the boiling point of water, at low moisture conditions, polysaccharides might also covalently link with phenolic compounds *via* Maillard reactions, forming, together with protein, high molecular weight nitrogenous brown compounds as reported for arabinogalactans during the roasting of the coffee (Moreira et al., 2015; Moreira et al., 2017). Ester, aryl-ether, stilbene type, and/or biphenyl linked phenolics are reported for these carbohydrate chimeric compounds (Coelho et al., 2014; Nunes & Coimbra, 2010). Polysaccharides can also be glycosidic linked to phenolic compounds *via* non-enzymatic transglycosylation reactions (Moreira et al., 2017), conferring antioxidant properties.

4.6. Contribution of adsorbed phenolics to polysaccharides antioxidant activity

Polysaccharides can interact with phenolic compounds (Fig. 2) by means of hydrophobic effects, hydrogen bonding and Van der Waals interactions (Fernandes et al., 2020; Liu, Le Bourvellec, & Renard, 2020). Although energetically weaker than covalent interactions, the occurrence of multiple binding sites along the polysaccharide backbone results in the formation of highly stable carbohydrate/phenolic complexes which dissociation might not be successful following conventional polysaccharide purification approaches as dialysis (Gonçalves et al., 2018) or even ethanol precipitation, as supported by the resilience of phenolic compounds to dissociate from cell wall material when using 80 % ethanol solutions (Le Bourvellec, Guyot, & Renard, 2004). Polysaccharide ethanol precipitation is in fact a polyphenol encapsulation procedure that takes advantage of polysaccharides chain aggregation for phenolic compounds entrapment (Munin & Edwards-Lévy, 2011). In this context, the antioxidant activity of polysaccharide rich fractions might still have a contribution from remnant phenolic compounds or other reducing agents that occur as contaminants, leading to misleading structure/function relationships. This has been shown for fucoidans

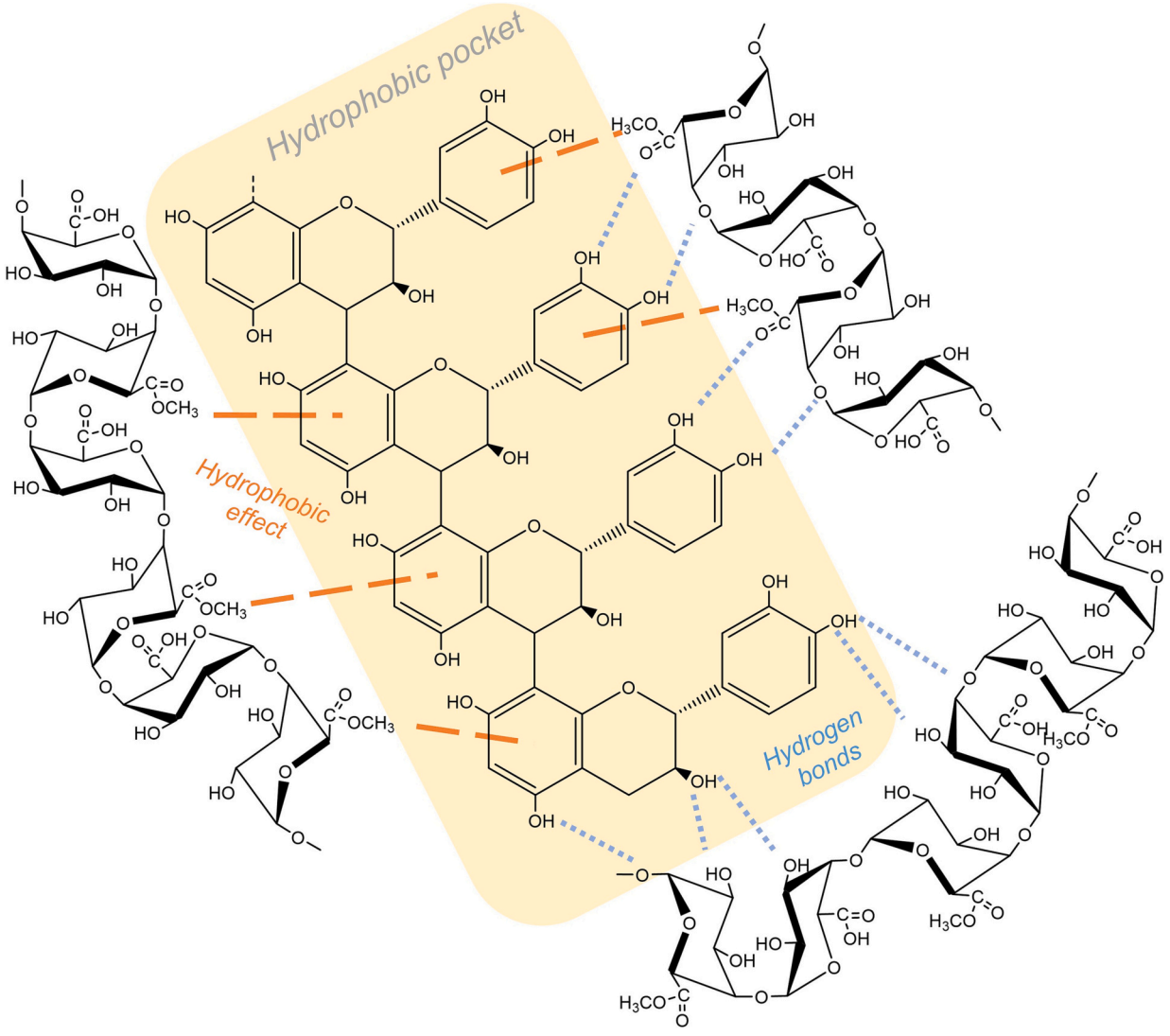


Fig. 2. Schematic representation of the mechanisms involved on the interactions between phenolic compounds, herein represented by procyanidins, and homogalacturonans.

which purification following ion exchange chromatography (Schneider et al., 2015) or phenolic compounds oxidation by H_2O_2 (Lahrsen et al., 2018), resulted in a decrease of the polysaccharides antioxidant activity and loss of its biological anti-cancer actions as tested in Raji cells, a Burkitt lymphoma cell line.

4.7. Contribution of covalently bonded proteins to polysaccharides antioxidant activity

The antioxidant activity of polysaccharides containing protein moieties can be also related to the reducing power of the aromatic amino acids tyrosine, phenylalanine and tryptophan, sulfur amino acids as cysteine and methionine and imidazole-containing amino acid histidine (Jaehrig, Rohn, Kroh, Fleischer, & Kurz, 2007; Wan et al., 2021). For instance, in type II arabinogalactan, tyrosine accounts for the main amino acid conferring antioxidant activity (Majee et al., 2016) while tryptophan contributes to the antioxidant activity of mannoproteins (Jaehrig et al., 2007).

4.8. Antioxidant activity of negatively-charged oligosaccharides

Lower molecular weight and negatively charged structures present the highest antioxidant activity as shown for oligosaccharides derived from pectic polysaccharides (2–6 kDa) (Yeung, Kang, So, Jung, & Chang, 2021), alginate (<6 kDa) (Zhao et al., 2012), κ-carrageenan (<2 kDa) (Sun et al., 2015), agar (<2 kDa) (Xu et al., 2018), short chain polysaccharides isolated from ulvans (28–151 kDa) (Qi et al., 2005), and mannoproteins (<75 kDa) (Galinari et al., 2018). This trend, as reported for chitosan, is associated with polysaccharides solubility restrictions. The process used for polysaccharide depolymerization also impacts the antioxidant activity of oligosaccharides. Acid hydrolysis of sulfated polysaccharides can result in the loss of sulfate groups in agar (Xu et al., 2018) and κ-carrageenan (Sun et al., 2015). In the case of pectin, the depolymerization *via* β-elimination reactions, as those promoted by pectin lyase, forms a double bond between C4 and C5 of the GalA methyl-ester at the non-reducing end of the generated fragments (Fig. 3) (BeMiller, 1986; Voragen, Coenen, Verhoef, & Schols, 2009) which might improve the antioxidant properties (Falkeborg et al., 2014).

5. Cellulose, starch, and other neutral polysaccharides

5.1. Neutral polysaccharide characteristics and antioxidant activity

Neutral polysaccharides have also antioxidant properties towards $OH^{\bullet}$, although less effectively than charged polysaccharides (Hu et al., 2016). A possible explanation outcome from the fact that, unlike negatively charged polysaccharides, neutral polysaccharides have low metal chelation properties (Hu et al., 2016; Machová & Bystrický, 2013; Rendleman, 1978), forming preferentially coordination complexes of less energetical stability that are still favorable to $OH^{\bullet}$ radical generation *via* Fenton reactions (Pristov et al., 2011). Cellulose (Pristov et al., 2011) and starch polysaccharides (Yang et al., 2010), the most common glucans in plants, are reported to form coordination complexes, mainly with Fe^{2+}, and with Cu^{2+} (Pristov et al., 2011; Saalwächter et al., 2000). These polysaccharides are also able to scavenge $OH^{\bullet}$, by oxidation of any of the ring carbons (Pristov et al., 2011). This occurs by hydrogen atom transfer mechanisms of the C—H hydrogen atom and, to a lower extent, the O—H one (Hernandez-Marin & Martínez, 2012).

The differences on the conformation of the anomeric carbon, β configuration for cellulose and α for starch, which is composed by two main polysaccharides, the unbranched or slightly branched amylose, and the highly branched amylopectin, yields distinct antioxidant properties. Contrarily to most polysaccharides, cellulose is more selective towards Cu^{2+} than Fe^{2+} (Pristov et al., 2011; Saalwächter et al., 2000). This difference may be due to the axial orientation of the Glc hydroxyl groups of cellulose, which favors the establishment of intermolecular hydrogen bonding, rendering a high compact and water insoluble structure (Saalwächter et al., 2000). Similarly, starch polysaccharides are also found as insoluble components when found as part of plant granules (Obadi, Qi, & Xu, 2023). However, starch solubilization upon gelatinization, providing a higher availability of functional groups, is expected to confer higher antioxidant properties to these polysaccharides when compared with cellulose.

Unlike cellulose, many other β-glucans are soluble in alkali solvent or aqueous solutions (Zhu, Du, & Xu, 2016). Water soluble derivatives of curdlan, a bacterial glucan composed of →3)-D-Glc*p*-(β1→ units, is reported to have higher antioxidant activity in Fenton like systems than laminaran, an algae glucan containing →3)-D-Glc*p*-(β1→ and →6)-D-Glc*p*-(β1→ in a proportion of 3:1, and lichenan, a plant glucan composed

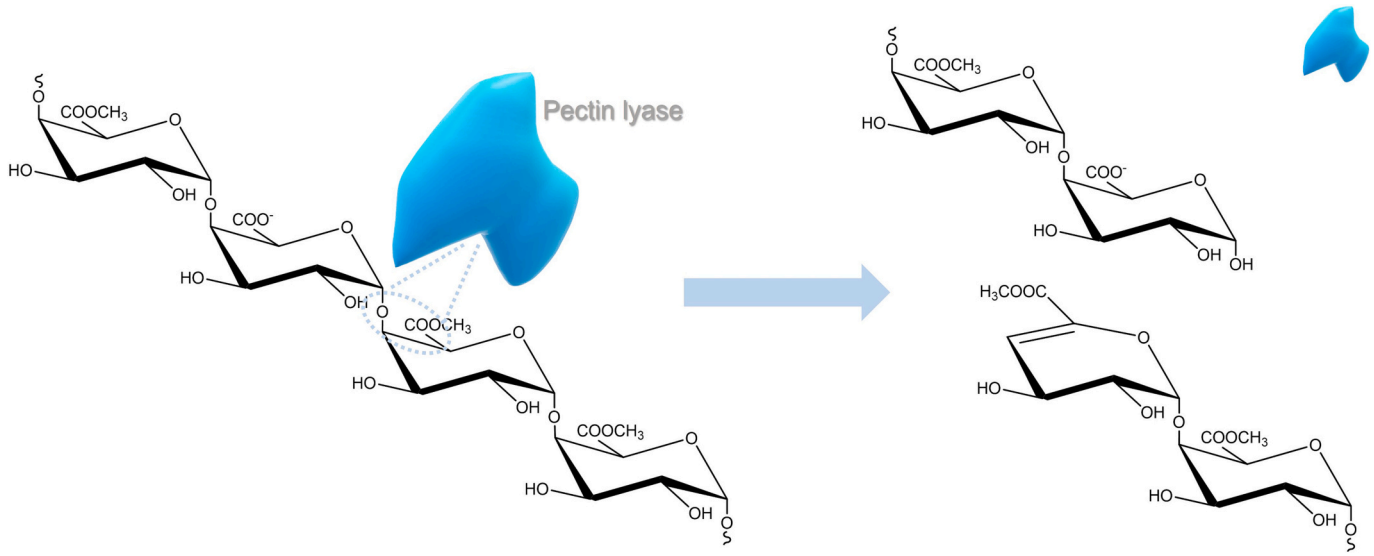


Fig. 3. Schematic representation of the generation of oligosaccharides containing a double bond between C4 and C5 of the GalA unit when following homogalacturonan depolymerization with pectin lyase or endo-pectin lyase, *via* β-elimination reactions. The non-esterified GalA units might also follow an eliminase mechanism by the action of endo- and exo-polygalacturonate lyases. There are also lyases that can generate these oligosaccharides from alginate, hyaluronic acid, and sulfated polysaccharides (Linhardt, Galliher, & Cooney, 1987).

of →3)-D-Glc*p*-(β1→ and →4)-D-Glc*p*-(β1→ units in a proportion of 1:2 (Machová & Bystrický, 2013). These data support the hypothesis that the occurrence of →3)-D-Glc*p*-(β1→ linkages have a positive impact on the antioxidant activity of polysaccharides due to the stabilization of radicals and/or promoting the formation of coordination complexes with Fe^{2+}. $OH^{\bullet}$ scavenging has also been reported for xyloglucans, with →4)-D-Glc*p*-(β1→ backbone branched with branched at O-6 with Xyl residues or short chain oligosaccharides composed of Fuc, Gal and Xyl (Tabbì, Fry, & Bonomo, 2001), galactomannans with a →4)-D-Man*p*-(β1→ backbone branched with D-Gal*p*-(α1→ (Cao & Ikeda, 2009; Hu et al., 2016; Pristov et al., 2011) and glucomannans with →4)-D-Man*p*-(β1→ and →4)-D-Glc*p*-(β1→ units (Hu et al., 2016).

5.2. Contribution of phenolics and other compounds to neutral polysaccharides antioxidant activity

The antioxidant activity of neutrally charged polysaccharides as measured by the $ABTS^{\bullet+}$, $DPPH^{\bullet}$, $O_2^{\bullet-}$ and many other radicals, given the lack of reducing power of the carbohydrate moiety (Hu et al., 2016; Siu et al., 2014), is ascribed to the occurrence of covalently linked phenolic compounds. For instance, the cellulose isolated from plant materials usually contains residual amounts of lignin derived compounds unable to be removed during cellulose purification steps based on high pressure and high temperatures treatments with aqueous alkaline, neutral or acidic solutions applied (Johansson, Campbell, Koljonen, & Stenius, 1999; X. Lu, Gu, & Shi, 2022). As lignin account interlinked phenolic structure composed of syringyl, guaiacyl and *p*-hydroxyphenyl units (Johansson et al., 1999), it confers to cellulose of plant origin a higher antioxidant activity than that of microbial origin, where lignin is absent (Pandit & Kumar, 2021). Oxidation reactions might also cause cellulose and xyloglucans to present covalently lined phenolic compounds (Fernandes, Le Bourvellec, et al., 2019). In the case of xyloglucans isolated from fruit pomaces, the phenolic moiety establishes a linking bridge with pectic polysaccharides (Fernandes, Silva, et al., 2019), which negatively charged GalA might also contribute to the overall antioxidant activity usually attributed to this complex. Covalently linked phenolic compounds can also be found by means of transglycosylation and esterification reactions as is the case of roasted coffee mannans and arabinogalactans (Moreira et al., 2015; Moreira et al., 2017). Maillard reactions are also prone to occur while following extraction processes that use very high temperatures, as is the case of microwave superheated water extraction (Ahmad & Langrish, 2012), often used to obtain higher yields of coffee mannans (Passos, Moreira, Domingues, Evtuguin, & Coimbra, 2014), yeast β-glucans (Reis et al., 2023) and many other neutral polysaccharides, resulting in antioxidant artifacts.

The occurrence of adsorbed phenolic compounds to oat β-glucans, composed of →3)-D-Glc*p*-(β1→ and →4)-D-Glc*p*-(β1→ backbone, is also reported to be responsible for the capability of β-glucan extracts to reduce iron and to scavenge $O_2^{\bullet-}$ and $DPPH^{\bullet}$, properties absent in the pure polysaccharide (Wu et al., 2011). In this sense, similar antioxidant artifacts can also be assigned to water soluble derivatives of xyloglucans (Park & Cosgrove, 2015), starches (Amoako & Awika, 2019; Oladele, Duodu, & Emmambux, 2020), brown algae laminarans (Mekoue Nguela, Poncet-Legrand, Sieczkowski, & Vernhet, 2016) and many other neutral polysaccharides. Like cereal β-glucans (Li et al., 2019), they acquire a coiled shape conformation of high flexibility, when in solution, suitable for phenolic compounds entrapment by means of non-covalent interactions.

Besides phenolic compounds, phytate and many other plant secondary metabolites retained by the carbohydrate backbone contribute to antioxidant effect of polysaccharide extracts (Wang, Maina, Ekholm, Lampi, & Sontag-Strohm, 2017). These interferences can also be observed in *in vitro* cellular models, where polysaccharide purification by ionic and size exclusion chromatography, allowing adsorbed phenolic compounds removal, resulted in the loss of their antioxidant effects in PC12 cell cultures against H_2O_2 (Siu et al., 2014). The protein fraction present in neutral polysaccharide extracts, as for instance β-glucans isolated from mushrooms (Liu et al., 2020; Liu, Le Bourvellec, & Renard, 2020) and yeast (Jaehrig et al., 2007), also contribute to the polysaccharides antioxidant activity. In the case of yeast, this protein fraction outcomes from short mannoprotein chains to which β-glucans are attached (Bastos et al., 2022).

5.3. Antioxidant activity of neutral oligosaccharides

When compared to neutral polysaccharides, oligosaccharides possess higher antioxidant properties (Siu, Xu, Chen, & Wu, 2016). The higher antioxidant properties are ascribed to structures obtained from radical depolymerization approaches given the introduction of carboxylic and other reducing groups (Choi et al., 2009), as described for acidic polysaccharides.

6. Modification of polysaccharides

The high antioxidant activity of arabinoxylans and many other polysaccharides substituted with phenolic compounds has been instigating research for the graft of these antioxidants into polysaccharide structures in which they are not usually found. Chitosan (Liu, Pu, Liu, Kan, & Jin, 2017; Nunes et al., 2013), pectin (Karaki et al., 2016), and starch (Wen et al., 2016) grafted with hydroxycinnamic acids, are some examples in which this approach effectively improved their antioxidant properties, when applied in hydrophobic environments. For instance, the grafting of ferulic acids provided starch the capacity to scavenge DDPH in alcohol solutions (Wen et al., 2016). The fact that polysaccharides posing phenolic groups present a more hydrophobic behavior (Fernandes, Silva, et al., 2019) might result on the improvement of their solubility in less polar solvents, contributing, besides the phenolic moieties, to the higher antioxidant activity of phenolic grafted polysaccharides.

To improve the antioxidant activity, polysaccharides have also been subjected to chemical modifications following sulfation (Xu et al., 2019), phosphorylation (Xia et al., 2021) and carboxymethylation (Chakka & Zhou, 2020) processes. High levels of substitution with carboxymethyl (Machová & Bystrický, 2013), sulfate (Wang et al., 2014) and phosphate (Xia et al., 2021) groups is also positively correlated with polysaccharides antioxidant activity. These groups provide polysaccharides an increased surface negative charge, causing chain expansion and intermolecular electrostatic repulsions, rendering the polymer high water solubility (Kagimura et al., 2015; Xia et al., 2021; Zhang, Zhang, Wang, & Cheung, 2003). This, together with the chelating properties characteristic of negatively charged polysaccharides (Musarurwa & Tavengwa, 2020), result on improved antioxidant properties, as reported for carboxymethylated →6)-D-Glc*p*-(β1→ glucans isolated from the *Lasiodiplodia theobromae* ascomyceteous fungus (Kagimura et al., 2015) and sulfated ones from edible *Ganoderma atrum* mushrooms (Zhang et al., 2015). Carboxymethylation in the C-2 amino group of chitosan also switches the ionic character of the amine from positive to negative (Musarurwa & Tavengwa, 2020). If partially substituted, carboxymethyl-chitosan can present amphoteric properties, amplifying the pH ranges at which chitosan acts as a metal chelator. Carboxymethylated polysaccharides are also ascribed to scavenge $OH^{\bullet}$ through the α-methylene hydrogens of the carboxylmethyl group (Saiki et al., 2011). In this sense, for equimolar substitutions, carboxymethylated polysaccharides are likely better antioxidants than sulfated and phosphorylated derivatives, in which this mechanism is absent.

The antioxidant activity by metal chelation of phosphorylated polysaccharides is also compromised at high levels of phosphorylation. This outcomes from intermolecular cross-linkages *via* phospho-diester and, less frequently, triester linkages, that decrease the number of available groups and their capability to act as antioxidant in Fenton like systems (Wang, Yang, et al., 2014) where Fe^{2+} act as oxidant. The partial depolymerization of the polysaccharides backbone that usually occurs

given the harsh conditions used during polysaccharides phosphorylation and sulfonation (Xu et al., 2019), positively impacts polysaccharide antioxidant properties.

The substitution with hydrophobic benzoyl and acetyl groups is also reported to ascribe improved antioxidant properties of polysaccharides as is the case of fucoidan (Wang et al., 2009) and ulvans (Qi et al., 2006). The high $OH^{\bullet}$ scavenging of benzoylated polysaccharides than those presenting acetyl groups can be attributed to the unpaired electrons of benzoyl aromatic ring, favorable for radical scavenging and metal chelation (Qi et al., 2006; Zhang et al., 2009). In opposition, the improvement on the antioxidant activity described for acetylated polysaccharides is likely to be related to a partial depolymerization during the acetylation process (Song et al., 2013). This is supported by the fact that highly acetylated polysaccharides have lower reducing power than non-substituted ones (Fuso et al., 2023), a feature likely due to the unavailability of an hydroxyl hydrogen atom ascribed to be involved in the antioxidant features of polysaccharides.

The antioxidant activity of polysaccharides against $OH^{\bullet}$ activity and many other radical species can also be improved by selenylation processes, yielding polysaccharide selenites with C-6 ester-linked selenious acid. This improvement is assigned to the selenium oxidation from Se^{4+} to Se^{6+} (Wei et al., 2015), and to polysaccharides partial depolymerization during the strong acidic conditions used during selenylation. In this context, polysaccharide selenites should have higher radical scavenging activity than native polysaccharides and those with substitutes of higher oxidation states as sulfate derivatives (Cao & Ikeda, 2009).

7. Concluding remarks

A critical deconstruction of polysaccharides antioxidant properties is presented here. Polysaccharides, regardless of their origin, might scavenge radicals or inhibit their formation by means of metal chelation. According to the literature survey, it was possible to identify that the polysaccharides *in vitro* chemical antioxidant action is highly dependent on their solubility, sugar ring structure, molecular weight, occurrence of positive or negatively charged groups, protein moieties and covalently linked phenolic compounds (Fig. 4). The latter two structural features have the higher contribution for polysaccharides antioxidant activity, allowing them to scavenge $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and even present metal reducing properties that pure polysaccharide fractions, devoid of phenolics and proteins, usually lack. The occurrence of phenolic compounds and protein is also an issue of concern when claiming polysaccharides as antioxidants given that their occurrence might be an artifact from sample manipulation, extraction conditions or even as contaminants not removed by most, polysaccharide purification approaches used. Their occurrence is likely to lead to misleading results in *in vitro* chemical methodologies often used during screening and characterization purposes, as well as *in vitro* biological methodologies and *in vivo* models. This should be considered when polysaccharide fractions are selected to be tested in both *in vitro* and *in vivo* experiments.

The information herein reviewed highlights the relevance of the establishment of structure/function relationships with possible antioxidant properties of polysaccharides and their use in food, pharmaceutical, and material sciences. To achieve this, future research should follow strategies that completely mitigate the bias provided by other compounds on polysaccharide antioxidant features. This should include the removal of polyphenols using aqueous/organic solvents in which polysaccharides are insoluble, preventing their occurrence as contaminants during polysaccharide extractions. Purification processes that effectively assure polyphenol, protein, and other contaminants removal, as is the case of solid-phase extraction or chromatographic techniques, should be followed and complemented with detailed characterization to identify the structural features modulating the antioxidant effects and avoid biased structure/function relationship assignments. This includes the assessment of polyphenol and proteic part constituent of the polymer. Only this strategy can pave the way for the development of novel carbohydrate design methodologies able to improve polysaccharides antioxidant effectiveness, currently 1000-fold-lower than the classical antioxidant active compounds. Until then, and despite falling in the concept of antioxidants given their propensity to inhibit oxidation

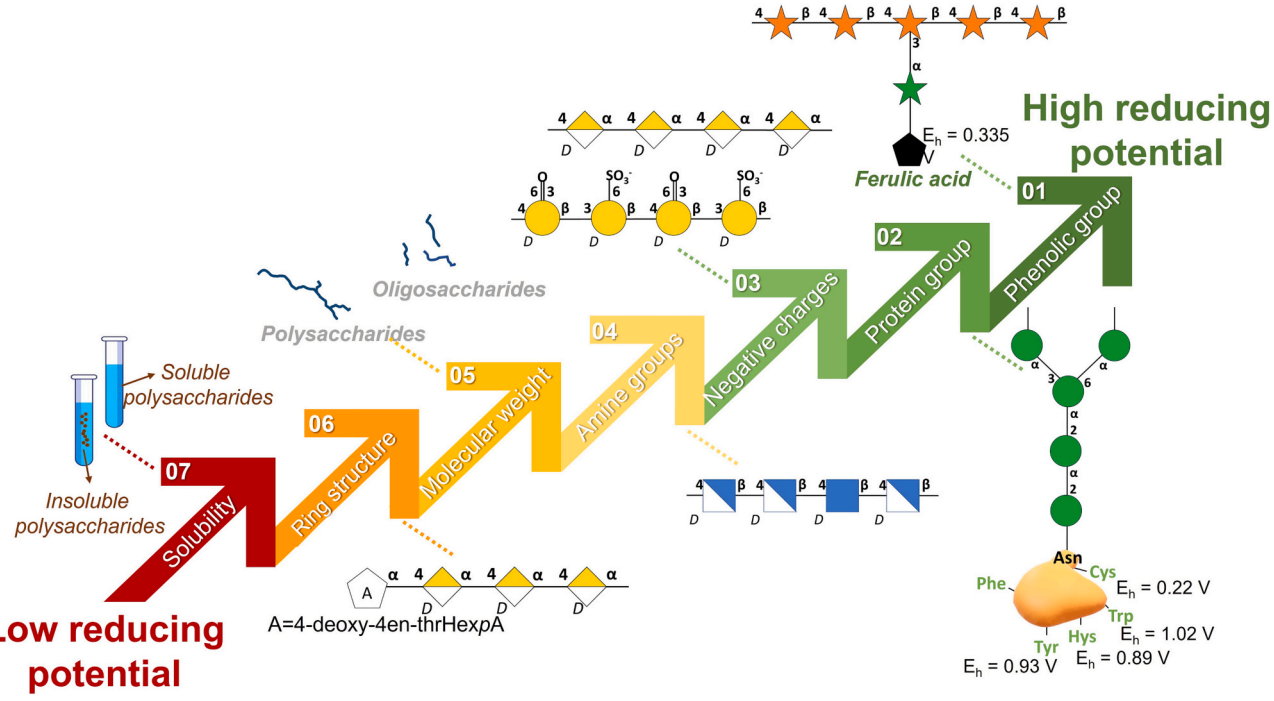


Fig. 4. Proposed hierarchy of the main features governing polysaccharides antioxidant properties. Herein are also represented some polysaccharide examples and the redox potential of the main substituents responsible for their antioxidant activity: ferulic acid (Teixeira, Gaspar, Garrido, Garrido, & Borges, 2013) found in arabinoxylans; cysteine (Cys) (Jocelyn, 1967), tryptophane (Trp) and tyrosine (Tyr) (Harriman, 1987), and histidine (Hys) (Medvidović-Kosanović, Stanković, Jozanović, Drulak, & Ilić, 2018), amino acid components of mannoproteins and many other proteoglycans.

reactions, the naming of polysaccharides as antioxidants must be prudently applied only accounting their chemical features and in well-established circumstances. This would allow to better use and develop strategies using polysaccharides for biomedical applications (Geng et al., 2023), for cosmetics (Albuquerque et al., 2022), food packaging and preservation (Saberi Riseh, Vatankhah, Hassanisaadi, & Kennedy, 2023), and food supplements (Qiu et al., 2022).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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