Send Orders for Reprints to reprints@benthamscience.net

Current Pharmaceutical Design, 2020, *26*, 1759-1777

REVIEW ARTICLE

An Overview of Structural Aspects and Health Beneficial Effects of Antioxidant Oligosaccharides

Tatiane F. Vieira[1], Rúbia C. G. Corrêa[2,3], Rosely A. Peralta[4], Regina F. Peralta-Muniz-Moreira[5], Adelar Bracht[1,6] and Rosane M. Peralta[1,6,*]

[1]*Program Post-graduated of Food Science, Universidade Estadual de Maringá, Maringá, PR, Brazil;* [2]*Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal;* [3]*Program of Master in Science, Technology and Food Safety, Cesumar Institute of Science, Technology and Innovation (ICETI), Centro Universitário de Maringá, Maringá, Paraná, Brazil;* [4]*Department of Chemistry, Universidade Federal de Santa Catarina, SC, Brazil;* [5]*Department of Chemical and Food Engineering , Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil;* [6]*Department of Biochemistry, Universidade Estadual de Maringá, Maringá, PR, Brasil*

Abstract: ***Background*:** Non-digestible oligosaccharides are versatile sources of chemical diversity, well known for their prebiotic actions, found naturally in plants or produced by chemical or enzymatic synthesis or by hydrolysis of polysaccharides. Compared to polyphenols or even polysaccharides, the antioxidant potential of oligosaccharides is still unexplored. The aim of the present work was to provide an up-to-date, broad and critical contribution on the topic of antioxidant oligosaccharides.

***Methods*:** The search was performed by crossing the words *oligosaccharides* and *antioxidant*. Whenever possible, attempts at establishing correlations between chemical structure and antioxidant activity were undertaken.

***Results*:** The most representative *in vitro* and *in vivo* studies were compiled in two tables. Chitooligosaccharides and xylooligosaccharides and their derivatives were the most studied up to now. The antioxidant activities of oligosaccharides depend on the degree of polymerization and the method used for depolymerization. Other factors influencing the antioxidant strength are solubility, monosaccharide composition, the type of glycosidic linkages of the side chains, molecular weight, reducing sugar content, the presence of phenolic groups such as ferulic acid, and the presence of uronic acid, among others. Modification of the antioxidant capacity of oligosaccharides has been achieved by adding diverse organic groups to their structures, thus increasing also the spectrum of potentially useful molecules.

***Conclusion*:** A great amount of high-quality evidence has been accumulating during the last decade in support of a meaningful antioxidant activity of oligosaccharides and derivatives. Ingestion of antioxidant oligosaccharides can be visualized as beneficial to human and animal health.

ARTICLE HISTORY

Received: October 11, 2019
Accepted: December 03, 2019

DOI:
10.2174/1381612824666180517120642

Keywords: Antioxidant, circular economy, functional properties, oligosaccharides, oligosaccharide derivatives, oxidative stress.

1. INTRODUCTION

There is now strong belief, corroborated by sound scientific research, that antioxidants own properties that are beneficial to people suffering from a series of illnesses. A massive number of investigations prove their essentialism in daily diets. Accordingly, antioxidants have always drawn the attention of the scientific community, though much more intensely in recent years [1-8]. Worldwide expert panels from various scientific fields show the action of both natural and synthetic antioxidants against a number of ailments such as cancer [9], cardiovascular dysfunction [10], neurodegenerative diseases and diabetes, in addition to inflammation [11] and aging [7].

The classic definition of Halliwell and Gutteridge [12] states that antioxidant is "a substance that, when present at a low concentration compared with that of an oxidizable substrate in the medium, inhibits oxidation of the substrate". In a simplified way, an antioxidant compound is a molecule able of inhibiting the oxidation of other molecules. Oxidation reactions can form free radicals, which in turn are atoms, molecules or ions with unpaired electrons, highly unstable and active towards chemical reactions with other molecules. Reactive oxygen species (ROS) comprising superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical (OH·), peroxyl (RO_2·), hydroperoxyl (HO_2·), alkoxyl (RO·), peroxyl (ROO·), nitric oxide (NO·), nitrogen dioxide (NO_2·), and lipid peroxyl (LOO·) and the non-radicals hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1\Delta_g$), and lipid peroxide (LOOH), are free radicals that provoke oxidative stress, causing devastating and irreversible damage to cell components (lipids, proteins and DNA) and various pathologies [8]. Essentially, antioxidant substances counteract the harmful activity of ROS in cell membranes by means of (1) hydrogen atom transfer; (2) single-electron transfer, and (3) the capability to chelate transition metals [10, 13]. Notwithstanding that normal cells possess antioxidant defense systems against ROS, including enzymatic and non-enzymatic systems, the extended accumulation of cell damage generates ailments such as cancer and accelerated aging [7]. Hence, the daily dose of antioxidant com-

*Address correspondence to this author at the Program Post-graduated of Food Science, Universidade Estadual de Maringá, Maringá, PR, Brazil; Tel/Fax: 55-44-30114715; E-mail: rmperalta@uem.br; rosanemperalta@gmail.com

1873-4286/20 $65.00+.00 © 2020 Bentham Science Publishers

Current Pharmaceutical Design

pounds plays an essential preventive role in oxidative stress-related illnesses as it counteracts the ROS damaging effects [6].

Antioxidants can be classified in manifold ways. Depending on their activity, they can be categorized as enzymatic and non-enzymatic antioxidants [14] (Fig. **1**). With respect to enzymatic antioxidants, they are sub-categorized into primary and secondary enzymatic defenses. The triad of first-line defense enzymes, namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), plays a vital role in the entire antioxidant defense strategy, particularly in what regards the superoxide anion radical ($O_2^{\cdot-}$) that is constantly produced by the normal body metabolism, mostly *via* the mitochondrial energy production pathway [15]. The secondary enzymatic defense, consisting of glutathione reductase and glucose-6-phosphate dehydrogenase, does not neutralize free radicals directly; however, this system has supporting roles for the other endogenous antioxidants [2]. Non-enzymatic antioxidants act by ceasing free radical chain reactions [14]. Fig. (**1**) displays the major classes of non-enzymatic antioxidants, as well as the most representative compounds of each class.

The other way of categorizing antioxidant compounds is based on their source. The antioxidants can be categorized as endogenous or exogenous antioxidants [13]. There is quite a number of non-enzymatic endogenous antioxidants, including vitamins (A), enzyme cofactors (Q10), in addition to low molecular weight molecules such as nitrogen compounds (uric acid), and peptides (glutathione) (Fig. **1**). In spite of its extraordinary efficiency, the endogenous antioxidant system does not attend to all of the body's demands, a reason why humans depend on several types of antioxidants from dietary sources to keep free radical concentrations at low levels. Vitamins (C and E), carotenoids (carotenes and xanthophylls), the wide group of polyphenols (simple phenols, phenolic acids, flavonoids, stilbenes, lignans and tannins), and antioxidant carbohydrates are all exogenous antioxidant compounds [2] (Fig. **1**). Furthermore, based on their polarity, antioxidant substances can be categorized in water-soluble (*e.g.* vitamin C) and lipid-soluble (*e.g.* vitamin E, carotenoids, and lipoic acid) antioxidants [13, 15].

In recent decades, research accomplishments in the field of natural antioxidants have expanded considerably the knowledge on naturally occurring compounds with beneficial health effects in foods [16]. Among these, vitamins, carotenoids and polyphenols [5, 6] were the most studied for their antioxidant activities, in particular the last group. However, lately, polysaccharides purified from natural products have given rise to an increasing interest in antioxidant carbohydrates [17] due to their promising pharmacological and biological activities [18, 19]. Likewise, promising antioxidant effects have been attributed to oligosaccharides, which are shorter-chain carbohydrates [20-22]. In the past ten years, the number of scientific articles regarding oligosaccharides has expressively increased, with an increment of more than 65% in the total number of publications (obtained from Web of Science, September 2019; period restricted to 2008-2018). Despite the growing interest in these bioactive compounds, oligosaccharides are not yet properly a hot topic in the field of antioxidant research. In the past five years, for instance, the papers holding the terms 'polysaccharide' *versus* 'antioxidant' (5060 publications) and 'polyphenol' *versus* 'antioxidant' (12423 publications) corresponded to more than 9-fold and 23-fold,

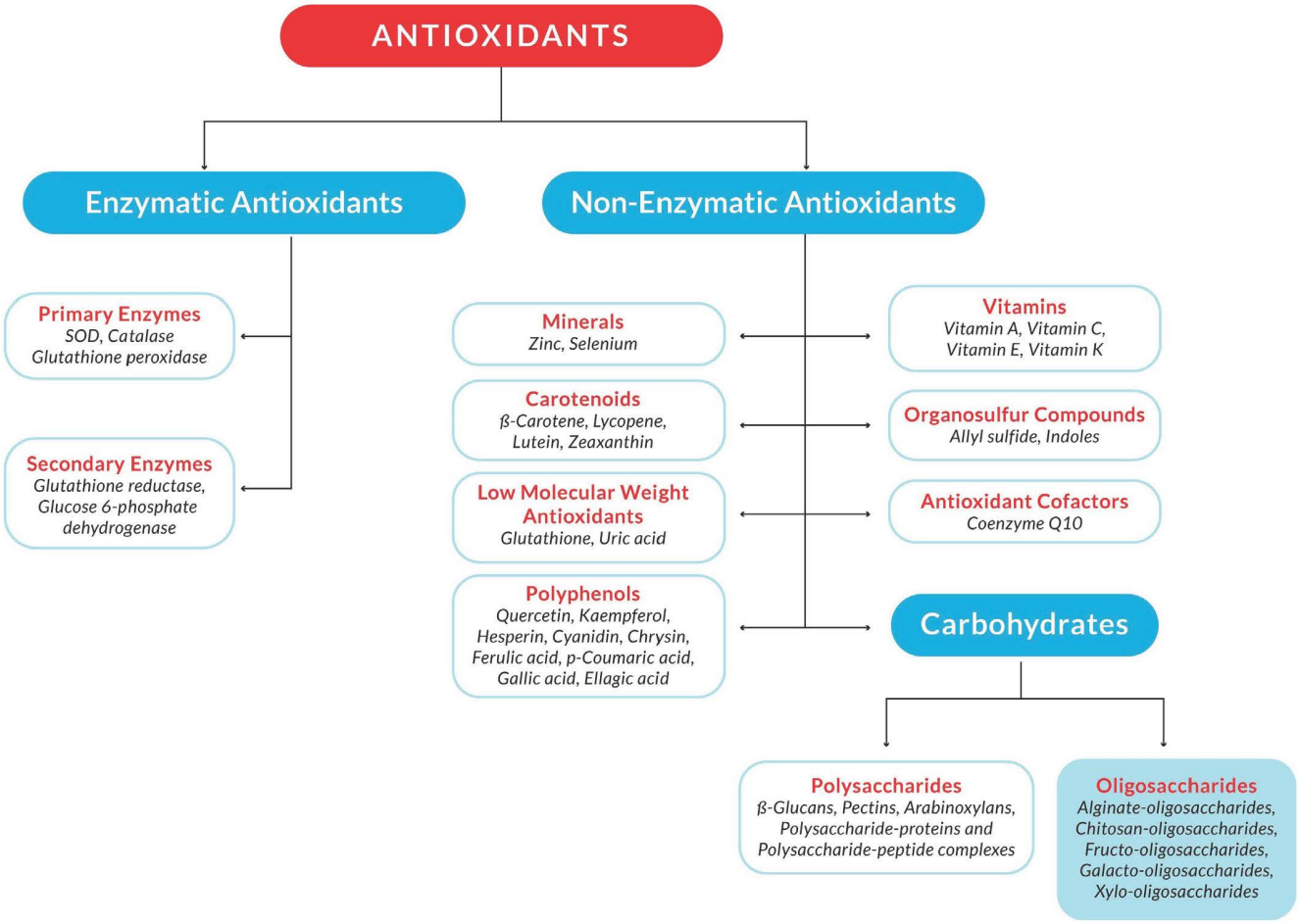

Fig. (1). Classes of natural antioxidant molecules.

respectively, the total number of reports containing the terms 'oligosaccharides' and 'antioxidant' (534 publications) (obtained from Web of Science, September 2019; keyword restricted to topics).

Bearing this in mind, the present report aims to provide an up-to-date, broad and critical contribution on the topic of antioxidant oligosaccharides, addressing the aspects involved in their obtainment and characterization, optimized production, evaluation of antioxidant potential, and therapeutic effects. With the purpose of presenting the latest advancements and trends in antioxidant oligosaccharides, most of the experimental reports were published after 2010.

2. OLIGOSACCHARIDES

2.1. Definition

Carbohydrates formed by one or two monomeric units are usually called monosaccharides and disaccharides, respectively. When the degree of polymerization is high, the term polysaccharide is applied. Polysaccharides may be composed of a great number of monosaccharide units, hundreds or thousands. When the degree of polymerization is relatively low, between 3 and 30 units, the term oligosaccharide is preferred [23]. Most of the few naturally occurring oligosaccharides are found in plants and play important roles such as carbon storage, translocation, and protection against stress caused by drought or low temperatures [24].

Based on their physiological fates in mammals or humans, the oligosaccharides can be classified as digestible or non-digestible. The concept of non-digestible oligosaccharides originates from the observation that the anomeric C atom (C1 or C2) of the monosaccharide units of some dietary oligo- and polysaccharides has a configuration that makes their corresponding glycosidic bounds resistant to hydrolysis by the digestive enzymes [25]. Fructooligosaccharides (FOS), the raffinose family of oligosaccharides (RFO), xylooligosaccharides (XOS) and isomaltulose, are the most widely distributed non-digestible oligosaccharides in the plant kingdom [26, 27]. Galactooligosaccharides (GOS) and fructooligosaccharides (FOS) are found in human milk [28, 29]. Several oligosaccharides are also commonly bound to lipids and amino acids through *O*-glycosidic and *N*-glycosidic bonds to constitute glycolipids and glycoproteins of many types of cells [30].

The non-digestible oligosaccharides gained notoriety as useful compounds for health preservation from the moment when they became to be considered, together with the non-digested polysaccharides, as prebiotics. Prebiotics are not hydrolysed by human digestive enzymes but they selectively enhance the activity of specific groups of beneficial bacteria, which are called probiotics. These bacteria can ferment prebiotics and produce short-chain fatty acids. The beneficial bacteria are present in the gut where they are known to promote the host's health by stimulating the immune system, inhibiting the growth of pathogenic bacteria and also by improving digestion and absorption of essential nutrients [31]. The reputation of prebiotic oligosaccharides as health promoters had a drawback due to their fermentation profiles and dosages required for health effects. Actually, most prebiotics belong to the group of dietary nondigestible carbohydrates, which includes resistant starch and resistant dextrins, non-starch polysaccharides, such as pectins, arabinogalactans, gum arabic, guar gum and hemicellulose, and nondigestible oligosaccharides such as inulin-type fructans, galactans, mannans, raffinose, and stachyose [32]. Xylooligosaccharides, pectinoligosaccharides, chitosanoligosaccharides, and agarooligosaccharides have received attention in recent years [33-35]. These molecules are known as "colonic foods" because they enter the colon and serve as substrates for the endogenous colonic bacteria, consequently providing the host with metabolic substrates, essential micronutrients, and energy. Of particular interest are prebiotics able to promote health benefits such as immunological activities that can promote the proliferation of beneficial bacteria and inhibit colonization of the gut by pathogenic ones, thus exerting a protective effect against acute or chronic gut disorders. Other benefits are their ability to lower blood cholesterol and to regulate glycemia, and their anti-tumour effects. In consequence, there is a growing interest in obtaining new low-cost prebiotics, useful for being used as food supplements, as well as in seeking for new *in vitro* and *in vivo* methods to evaluate their mechanisms of action. Several non-digestible oligosaccharides have been used in the food industry as prebiotic supplement products or food ingredients [36].

In general terms, it is believed that the regular consumption of prebiotics protects against the development of the so called Western diseases, including diabetes, cardiovascular disease, colon cancer, obesity, abnormal lipid metabolism and chronic inflammatory diseases [31, 32]. Oxidative stress plays an important role in the worsening of these pathological conditions. For this reason, at least in theory, the capability of oligosaccharides to act as antioxidants could have beneficial effects in maintaining good health.

2.2. Methods for Obtaining Oligosaccharides

Oligosaccharides can be obtained from natural sources and through chemical and/or biotechnological processes.

2.2.1. Extraction

Oligosaccharides can be found at various concentrations as natural components of milk, honey, sugarcane juice, fruits and vegetables such as chicory, asparagus, onions, garlic, bananas, leeks, rye, wheat, soybeans, mustard, bamboo shoots, Jerusalem lentils, yacon, barley, tomatoes, and artichoke. Generally, these natural sources contain concentrations ranging from 0.3 to 6% fresh weight. However, there are only a few classes of naturally produced oligosaccharides and because of their structural complexity, isolation from their corresponding sources is generally quite difficult [37, 38].

When oligosaccharides are naturally available in food, only the extraction process is required. This process can be performed by solubilizing the substrate in water, methanol or ethanol [39]. More recently, ultrasonic and microwave extractions have been used in the extraction of oligosaccharides from different plant materials. Ultrasonic extraction has been widely used because of its capillary effects since microwaves can penetrate the plant matrix and generate heat within the cells, both resulting in cell disruption and enhanced mass transfer [40, 41].

2.2.2. Obtainment of Oligosaccharides by Depolymerization of Polysaccharides

Oligosaccharides can be obtained from the depolymerization of polysaccharides such as starch, inulin, pectin, xylan, glucan, mannan, arabinan, galactan, chitosan, among others [32, 38, 40]. The depolymerization of polysaccharides can be performed by chemical, physical or enzymatic processes [38, 39]. Chemical and physical methods can be combined to increase the hydrolysis efficiency.

In chemical depolymerization, mineral acids (H_2SO_4 and HCl) and organic acids (such as maleic, oxalic, acetic or trifluoroacetic) are employed at different concentrations as catalysts [38]. This process, also called acid hydrolysis, is relatively simple, inexpensive and easy to control since the reaction is interrupted by neutralization of the medium [39]. In the alkaline depolymerization of xylans, NaOH, KOH, $Ca(OH)_2$, and ammonia can be used for obtaining xylooligosaccharides [42].

Hydrogen peroxide (H_2O_2) can also be used to depolymerize various polysaccharides. H_2O_2 is easy to handle, readily available, and environment-friendly [43]. This technique is based on the formation of free radicals, which can attack the glycosidic linkages of the polysaccharides.

In physical processes such as hydrothermal treatments or autohydrolysis, acids are generally not used and hydrolysis occurs due to the high temperatures [38]. The temperatures employed generally

range from 130 to 230 °C [39]. In this process, hydrogen ions (H_3O^+) derived from auto-ionization of water act as catalysts. In addition, partial cleavage of acetyl groups to acetic acid during the process results in increased H_3O^+ concentration in the reaction medium [38, 39]. Gamma radiation, ultraviolet light, microwaves, ultrasound, high-pressure dynamic micro-fluidization have also been applied to the production of oligosaccharides [39, 44]. Although physical processes represent a fast and clean way to produce oligosaccharides, their applications are still limited, and the reaction conditions must be optimized for better performance and for solving problems related to the basic kinetics of polysaccharide hydrolysis.

Enzymatic hydrolysis usually requires mild conditions (low temperature) that prevent the formation of sugar breakdown products such as furfural and 5-hydroxymethylfurfural. However, this process requires greater control and the use of different enzymes due to the structural diversity of polysaccharides and enzymatic stereo-specificity [38]. In the enzymatic processes, mixtures of oligosaccharides with different degrees of polymerization are formed. The chemical structures and composition of these mixtures depend on the type and source of enzymes and on the processing conditions, including the initial substrate concentration [32]. Depending on the initial substrate, the production of oligosaccharides may be accompanied by the production of monomers followed by the generation of disaccharides and other oligomers through the action of transferases and the reverse hydrolytic activity of the hydrolases [32, 45].

For breaking down the polysaccharide glycosidic bonds, different enzymes can be used. Glycoside hydrolases (EC 3.2.1.-) are a widespread group of enzymes which hydrolyse the glycosidic bond between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety. These enzymes catalyze hydrolysis by means of general acid catalysis that requires two critical residues: a proton donor and a nucleophile/base. This hydrolysis occurs through two mechanisms that give rise to a general retention or inversion of the anomeric configuration. As the polysaccharide structures are diverse in terms of monomeric structures as well as in terms of their glycosidic linkages, different polysaccharide hydrolases can be used in the enzymatic degradation of a polysaccharide for producing oligosaccharides. Some examples are β-mannanase, (EC 3.2.1.78), chitinase (EC 3.2.1.14), xylanase (EC 3.2.1.8), inulinase (EC.3.1.2.7), β-agarase (EC. 3.2.1.81), and pectinase (EC.3.21.15), which hydrolyze the glycosidic linkages of mannan, chitin, xylan, inulin and agar, respectively. Polysaccharide lyases (EC 4.2.2.-) cleave the glycosidic bonds of uronic acid-containing polysaccharides by a β-elimination mechanism to generate an unsaturated hexenuronic acid residue and a new reducing end at the point of cleavage [46, 47]. More information about these and other enzymes capable to depolymerize polysaccharides can be found in www.cazy.org.

2.2.3. Enzymatic Synthesis of Oligosaccharides

Glycosyl transferases (GTs, EC 2.4.x.y) can be used for enzymatic synthesis of oligosaccharides [48]. GTs are enzymes that catalyse the transfer of sugar moieties from activated donor molecules to specific acceptor molecules, forming glycosidic bonds *in vivo* [49]. Although GTs are good catalysts for oligosaccharide synthesis, their application is limited due to their low availability and the use of complex and expensive activated substrates. In addition, GTs are unstable in solution, which makes them unrealistic for industrial applications. These limitations lead to an increased use of transglycosylases (GHs) [48].

Some GHs may catalyze reverse hydrolysis (thermodynamic control) or transglycosylation (kinetic control) of the anomeric configuration, a retention mechanism that can lead to oligosaccharide synthesis (Fig. 2) [45, 49]. They are typically less regio-selective and the oligosaccharide production yield is lower than that one obtained by GTs [45]. However, they are more readily available, stable, easy to handle, require no cofactors and act on simple and inexpensive donors such as monosaccharides and disaccharides [48]. GHs are mainly responsible for the hydrolysis of short-chain oligosaccharides resulting from the synergistic action of endoglucanases producing free glucose as a rate-limiting step. An efficient catalyst for synthesis, but not hydrolysis, of glycosidic bonds can be generated by site-directed mutation of GHs [50, 51].

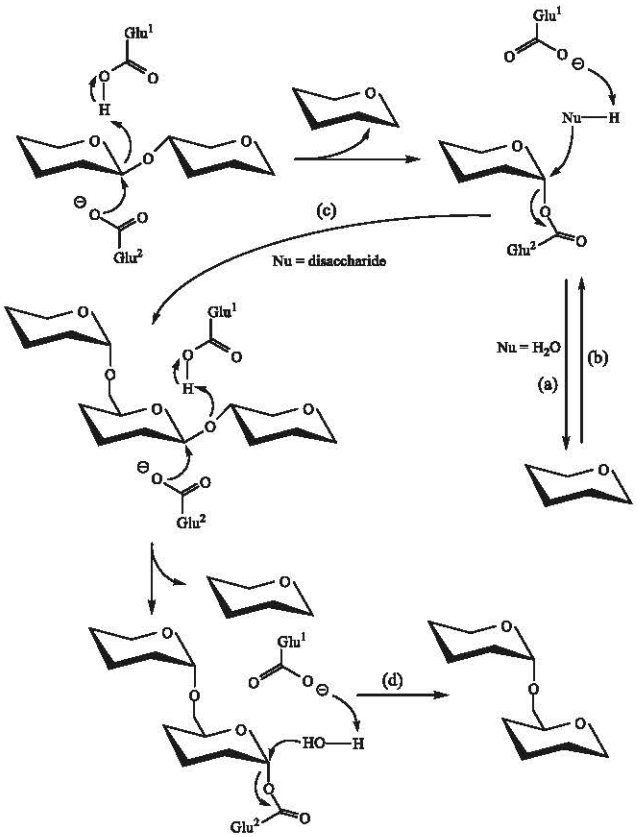

Fig. (2). Generalized mechanism of a transglycosylase. (**a**) Hydrolysis; (**b**) Reverse hydrolysis; (**c**) transglycosylation; (**d**) secondary hydrolysis; Glu^1, Glu^2: glutamate; Nu: nucleophile.

When a disaccharide is used as a substrate, the transglycosylation product is formed *via* self-condensation. For this to occur, the process must be faster than the hydrolysis of the glycoside; the enzyme transfers the glycosyl residue from the donor to an acceptor with retention of the anomeric configuration. The primary hydroxyl group reacts preferably in a way that leads to the formation of 1-6 bonds, although the bonding may occur at all positions, leading to a variety of different products. In order to direct the reaction to transglycosylation, the main strategy is to use high concentrations of substrate [49].

2.2.4. Chemical Synthesis of Oligosaccharides

Chemical synthesis can also be used for obtaining oligosaccharides. Continuous monitoring is mandatory in this type of synthesis because it usually requires the use of pure and hazardous chemicals, making it expensive and laborious. The process involves strategies of protection, deprotection, and activation to control the regioselectivity and stereochemistry of the resulting oligosaccharide. These strategies are undesirable and unrealistic for large scale production and result in low yields [48]. On the other hand, in enzymatic synthesis, orthogonal protection/deprotection of the different portions is not necessary due to the excellent regio-

selectivity of enzymes and because of the full stereo-chemical control of the new binder [27,48,49].

2.2.5. Quantification and Structural Analysis of Oligosaccharides

Establishing structure-function relationships is important for elucidating biochemical mechanisms of action. However, due to the possibility of a large number of carbohydrate isomers for a given chemical formula, the structural identification is still a difficult task [52]. An oligosaccharide containing six hexoses, for example, has more than 1012 possible isomers, and differentiation of such a large number of isomers using a single and simple analytical method becomes difficult [53]. Furthermore, most naturally occurring oligosaccharides and those generated from polysaccharide hydrolysis or chemical and enzymatic synthesis exist in mixtures of various complexities [36]. The complexity of the mixtures often requires the use of a consortium of different analytical techniques for complete chemical characterization [54, 55]. In addition, purification is always a prerequisite prior to structural analysis [36].

The absence of chromophore groups in many oligosaccharides makes detection problematic. Thus, derivatization of oligosaccharides is indispensable to achieve highly sensitive detection [56]. Some common derivatization strategies include reductive amination, permethylation and hydrazide labeling of the reducing end of the oligosaccharide [57].

Colorimetric methods, such as the 3,5-dinitrosalicylic acid assay, which detect reducing ends of oligosaccharides, can be used for quantification [54, 55]. However, techniques that provide qualitative and quantitative information of independent oligosaccharides such as planar chromatography, gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (EC) are the most widely used and can be coupled to spectroscopic instruments for structural information [55-57].

Among the planar chromatographic techniques, thin layer chromatography (TLC) is the most common method in the characterization of oligosaccharides. When compared to HPLC, it is less efficient in the separation of complex mixtures. However, TLC also offers some benefits: it is simple and adaptable to equipment availability and does not require specially trained technicians, being available to all types of laboratories [58]. TLC and high-performance thin layer chromatography (HPTLC) have been largely used, for example, in the analysis of human milk oligosaccharides [59]. HPTLC was recently used in the analysis of xylooligosaccharides obtained from sugar cane bagasse [60].

Because oligosaccharides have high polarity and low volatility, the use of GC in principle does not seem ideal, since a previous derivatization step is often required [61, 62]. However, GC has proven to be the most appropriate technique in many cases, particularly when only small samples are available and/or oligosaccharides appear as a complex mixture of isomers [62]. GC coupled to mass spectrometry (MS), GC-MS, has been shown to be a valuable technique for the identification of unknown carbohydrates [61]. Recently, structural differences between pectin oligosaccharides (POS) obtained through enzymatic hydrolysis of pectins of various origins have been elucidated by GC-MS [63].

Traditional methods of HPLC can be combined with mass spectrometry (HPLC-MS) and also with amperometric, fluorescence and refractive index pulse detectors [64]. HPLC was used recently in studies with mannanoligosaccharides [65] and xylooligosaccharides [66].

Sophisticated techniques such as high-performance anion exchange chromatography (HPAEC) and high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) can be used to evaluate oligosaccharides at very low concentrations (femtomole, picomole) [35, 54, 67].

In terms of resolution, capillary electrophoresis (CE) is considered one of the most powerful techniques, being useful in the analysis of free oligosaccharides, glycoproteins, and glucosaminoglycans. As an example, it was recently used for the analysis of glucose_oligomers_in wort samples to follow the fermentation_process using *Saccharomyces pastorianus* and *Saccharomycodes ludwigii* yeast strains [68].

For the direct determination of oligosaccharide structure, nuclear magnetic resonance (NMR) and mass spectrometry (MS) techniques are widely used [53, 55]. MS is based on the conversion of components of a sample into gaseous ions, which are taken to a mass analyzer, where they are separated according to the mass/charge ratio [54]. Its application is challenging due to the low carbohydrate ionization efficiency of mass spectrometers and the similarity of mass spectra among large numbers of isomers [53].

As a standard approach, the molecular mass of an oligosaccharide is determined by MS, while the types of monosaccharide bindings and side group positioning are resolved by NMR, MS-GC and MC-MS/MS [69]. LC-MS has been considered especially useful in the characterization of neutral and acidic oligosaccharides, such as pectin oligosaccharides [54].

The structures of FOS, highly purified by means of high-speed counter-current chromatography (HSCCC) coupled with pre-column derivatization, were determined by mass spectrometry (MS) and nuclear magnetic resonance (NMR) [70]. Although widely used, NMR has low sensitivity and severe signal overlap, which often makes data interpretation difficult [71].

Recent studies have employed mass spectrometry associated with matrix-assisted laser desorption and ionization followed by detection on a flight time type analyzer (MALDI-TOF-MS) to analyze the oligosaccharides from longam [41], xylo-oligosaccharides from eucalyptus glucuronoxylan obtained by auto-hydrolysis [72], and chitosan oligosaccharides [73]. The most common methods used for oligosaccharide ionization are impact ionization of electrons (EI), electrospray ionization (ESI) and desorption ionization/matrix-assisted laser ionization (MALDI) [54, 61, 74, 75]. Recently, the xylooligosaccharides obtained by auto-hydrolysis of bamboo by ESI-MS [33], and the neoagarooligosaccharide from *Gracilaria* were characterized by ESI-TOF-MS [76]. Recently, the quadrupole time-of-flight tandem mass spectrometry (Q-TOF-MS/MS) was used directly to analyze the structures of oligosaccharides produced by the action of endo-β-1,3(4)-D-glucanase Eng16A from *Coprinopsis cinerea* on barley β-glucan [77] and products of the action of chitin deacetylases (Cda1 and Cda2) from the mushroom *Coprinopsis cinerea* on chitin oligosaccharides [78].

Vibrational spectroscopy techniques such as Infrared (IR) and Raman are also versatile, powerful and complementary tools for structural characterization of carbohydrates, including oligosaccharides. Both techniques provide spectra with a different set of characteristic bands and indicate, for example, in the IR spectra, the nature of the H bond, or the Raman spectra, the ring configuration [79]. Fourier-transform infrared spectroscopy (FTIR) is the most common technique used to characterize oligosaccharides. Recently, the raffinose family of oligosaccharides was characterized using, among other methods, FT-IR [80].

3. ANTIOXIDANT PROPERTIES OF OLIGOSACCHARIDES

Antioxidant activity can be analyzed by different *in vitro* and *in vivo* methods. Generally, *in vitro* antioxidant tests are easier to be executed [81]. *In vivo* tests allow analysis under physiological conditions, but require the use of animal models, some of which (such as mammals) are expensive and time-consuming.

3.1. *In vitro* Studies

Table **1** presents recent reports of investigations in which the obtainment of oligosaccharides was followed by an evaluation of their antioxidant properties by different *in vitro* methods.

Table 1. Antioxidant activities of oligosaccharides evaluated by *in vitro* methods.

Source and Obtainment	Most Important Observations and Conclusions	Refs.
Extraction		
Hot water extraction of oligo-saccharide from the mushroom *Hericium erinaceus*.	An oligosaccharide from the fruiting bodies was purified through chromatographic methods. The oligosaccharide is composed by D-xylose and D-glucose, and has a molecular weight of 1,877 Da. It presented antioxidant activity as evaluated by three methods: DPPH, ABTS and hydroxyl radical scavenging activities.	[82]
Extraction of an oligosaccharide from green asparagus.	The oligosaccharide was purified using Sephadex G-25 and presented a molecular weight of 569 Da. It was able to scavenge hydroxyl and superoxide radicals with a dose-effect relationship.	[83]
An oligosaccharide from longan pulp was extracted by an optimized ultrasonic-microwave method.	The purified oligosaccharide exhibited a dose-dependent scavenging activity of the 1,1-diphenyl-2-picrylhydrazyl radical.	[41]
From Degradation of Polysaccharides		
Pectin oligosaccharides were obtained from degradation of citrus pectin by irradiation (20 kGy).	Antioxidant properties of pectin oligosaccharides produced by irradiation were confirmed by two methods, DPPH scavenging activity and β-carotene–linoleic acid bleaching assay. The pectin oligo-saccharides were not mutagenic and inhibited growth of tumor cells.	[84]
Carrageenan is a collective term for a group of sulfated polysaccharides extracted from marine red algae. κ-Carrageenan oligosaccharides were prepared through acid hydrolysis of the polysaccharide followed by synthesis of their oversulfated, acetylated, and phosphorylated derivatives.	Superoxide anion, hydroxyl radical and DPPH free radical scavenging activities were used to evaluate the antioxidant activities of carrageenan and derivatives. The derivatives of carrageenan oligo-saccharides exhibited higher antioxidant activity than the poly- and oligosaccharides: the oversulfated and acetylated derivatives, which scavenged superoxide radicals, the phosphorylated and low-degree of sulfation acetylated derivatives, which scavenged hydroxyl radicals, and the phosphorylated derivatives, which scavenged DPPH radicals, all exhibited significant antioxidant activities. The effect of the molecular weight of the carrageenan on the antioxidant activities is not obvious, considering that both polysaccharide and the mixture of oligosaccharides exhibited a similar activity against the three antioxidant systems *in vitro*.	[85]
κ-Carrageenan oligosaccharides were obtained by the degradation of parent κ-carrageenan using free radical depolymerization, mild acid hydrolysis, κ-carrageenase digestion and partial reductive hydrolysis. The structure types were accurately and comparatively elucidated by ESI-MS and CID MS/MS.	The antioxidant activities of different degradation products were investigated by four different antioxidant assays, including superoxide radical scavenging activity, hydroxyl radical scavenging activity, reducing power and DPPH radical scavenging activity. The various depolymerization methods influenced the antioxidant activities of the κ-carrageenan oligosaccharides. These results indicate that the antioxidant activities of κ-carrageenan oligosaccharides could be related to the degree of polymerization, the content of reducing sugar and sulfate groups, and the structure of the reducing termini.	[86]
Degradation of a polysaccharide from the mushroom *Flammulina velutipes* with H_2O_2.	The resulting oligosaccharides showed strong hydroxyl radical scavenging activity and reducing capacity at the concentration of 100 μg/mL.	[87]
Oxidative degradation of xanthan under acidic and alkaline conditions produced two oligosaccharides XGOS-A (MW 7500 Da) and XGOS-B (MW 7330 Da), respectively.	The antioxidant activities of both oligosaccharides were inferred from their capacity in scavenging superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical (•OH), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and hydrogen peroxide (H_2O_2) and by measuring their ferrous ion chelating activity and reducing power. XGOS-B had a more pronounced antioxidant activity than XGOS-A. The antioxidant activity of the xanthan oligosaccharides appear to be related to their contents of pyruvic acid and the reducing sugar.	[88]
Maleoyl xanthan oligosaccharides (XGOSMA) and phthaloyl xanthan oligosaccharides (XGOSPA) were prepared by reacting xanthan oligosaccharides with maleic anhydride and phthalic anhydride, respectively.	The antioxidant activities of the xanthan oligosaccharide derivatives were deduced from their ability to scavenge the superoxide anion radical ($O_2^{\cdot-}$) and the hydroxyl radical (OH), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in addition to a determination of their reducing power. The results indicated that XGOSPA exhibited higher antioxidant activity than XGOSMA with similar substituting degrees in all the above mentioned antioxidant evaluation systems. This may be related to the fact that the phthaloyl group has a stronger electron-withdrawing effect than the maleoyl group.	[89]
Peach gum oligosaccharides were obtained by depolymerization of peach gum polysaccharide using H_2O_2.	Peach gum derived oligosaccharides presented high hydroxyl radical scavenging and high DPPH scavenging activities at a concentration of 100 μg/mL.	[90]

Table 1 contd....

Source and Obtainment	Most Important Observations and Conclusions	Refs.
N-Maleoyl chitosan oligosaccharide (NMCOS) and N-succinyl chitosan oligosaccharide (NSCOS) were prepared from a chitosan oligosaccharide by acylation with maleic anhydride and succinic anhydride, respectively.	The antioxidant activities of the derivatives were evaluated by superoxide anion O_2^{-} and hydroxyl radical OH scavenging and determination of reducing power. Results suggest that NMCOS possesses stronger antioxidant activities, which may be related to the fact that the maleoyl moiety has a stronger electron-withdrawing effect than the succinyl moiety.	[91]
A N-furoyl chitosan oligo-saccharide (NFCOS) was prepared *via* acylation of chitosan and chitosan oligosaccharide.	The derivative exhibited higher antioxidant activities than the chitosan oligosaccharide as determined by three methods: DPPH scavenging activity, reducing power and hydroxyl radical scavenging activity.	[92]
An oligosaccharide from flaxseed was obtained using the H_2O_2 oxidative method.	The flaxseed gum oligosaccharide (FGOS) was characterized by HPLC-SEC, GC, FTIR, NMR and FESEMl. FGOS exhibited good free radical scavenging ability ($OH^{\bullet}$ 82.58%, $DPPH^{\bullet}$ 52.74% and $ABTS^{\bullet}$ 91.29% at most, respectively), suggesting a potent antiradical activity.	[93]
Ultrasound irradiation and enzymatic hydrolysis were applied to the production of antioxidant xylooligosaccharides from wheat chaff.	The filtrate prepared by ultrasound pre-treatment of wheat chaff was evaluated for its antioxidant capacity using the ABTS radical-scavenging assay. The resulting tested activity was equal to 1.03 ± 0.01 μmol ascorbic acid equivalent/g.	[94]
The corn cob xylan was extracted using dilute acid, dilute alkali and sodium hypochlorite. The extracted xylan (XOS) was subjected to enzymatic hydrolysis using *Bacillus aerophilus* KGJ2 xylanase.	XOS was tested for its DPPH radical scavenging activity and presented a IC_{50} of 1 mg/ml.	[95]
The polysaccharide of *Crassostrea gigas* (→4)-α-d-Glc-(1→ with few →3,4)-β-d-Glc-(1→ and →2,4)-β-D-Glc-(1→branched units), a shellfish largely cultivated in China, was depolymerized using H_2O_2.	The oligosaccharides presented elevated antioxidant activities evaluated by two methods, hydroxyl radical scavenging (HRSA) and DPPH free radical scavenging activities.	[96]
Xylooligosaccharides (XOS) from beechwood and birchwood glucuronoxylans were produced by enzymatic hydrolysis using two xylanases, a GH10 (Xyn10A) and a GH30 (Xyn30D).	Xyn10A produced a mixture of neutral and acidic XOS and the XOS produced by Xyn30D were all acidic containing a methylglucuronic acid (MeGlcA) ramification.The substituted acidic XOS-MeGlcA showed a high and stronger antioxidant activity, determined as ABTS scavenging ability, than the XOS produced by Xyn10A . The antioxidant activity increased with the degree of polymerization of XOS, and depended on the type of xylan substrate used.	[72]
Crude *Aspergillus fumigatus* xylanase was used for hydrolysing wheat husk without any pre-treatments for producing XOS.	The scavenging ability of XOS obtained from the 12 h enzymatic hydrolysis was studied using the DPPH assay. The XOS exhibited concentration-dependent antioxidant activity, with a maximum of 74% at the concentration of 6 mg/ml.	[97]
Thermoascus aurantiacus family 10 endoxylanase (XYLI) was used to obtain feruloylated oligosaccharides from insoluble wheat flour arabinoxylan (WFAX).	A feruloyl arabinoxylotrisaccharide (FAX3) showed high antioxidant activity in the 2,2-diphenyl1-picrylhydrazyl (DPPH) reduction assay, exhibiting an antiradical efficiency, and inhibited the copper-mediated oxidation of human low density lipoprotein (LDL) in a dose-dependent manner with almost complete inhibition at 32 μM.	[98]
Agar (1–4)-linked 3,6-anhydro-α-l-galactose alternating with (1–3)-linked β-D-galactopyranose) was extracted from *Gelidium aman-sii* and hydrolysed by a recombinant β-agarase.	The oligosaccharides neoagaro-octaose and neoagaro-decaose exhibited increased radical scavenging activity towards 2,2-diphenyl-1-picrylhydrazyl and 2,2-azino-bis (3-ethylbenzothiazoline sulfonic acid) radicals.	[99]
Agaro-oligosaccharides were ob-tained from commercial agarose through an enzymatic hydrolysis reaction using cellulase from *Trichoderma reesei.*	Oligosaccharide samples were able to scavenge the. $ABTS^{+}$ and DPPH radicals, and also capable to reduce ferric tripyridyl-triazine (ferric ion reducing power).	[100]
Alginates with different guluronic (G) and mannuronic (M) acids were submitted to radiation-induced degradation in aqueous and H_2O_2 solutions.	Alginate oligosaccharides with molecular weights (MW) from 1000 to 3750 Da were obtained by γ-irradiation of NaAlg solution in the presence of small amounts of H_2O_2 at low doses (below 5.0 kGy) and by controlling the G/M.The antioxidant properties of the fractions with various molecular weights and different G/M ratios were evaluated by using the DPPH method. Both MW and G/M ratio are important factors in controlling the antioxidant properties of alginate oligosaccharides. Lower G/M ratios lead to relatively strong scavenging abilities as evaluated by the DPPH method.	[101]

Table 1 contd....

Source and Obtainment	Most Important Observations and Conclusions	Refs.
Alginate oligosaccharides (AOS) were prepared from alginate using alginate lyase. The AOs were structurally characterized by thin layer chromatography, infrared spectrometry, and mass spec-trometry. The AOs were structurally characterized as a mixture of dimers, trimers, and tetramers.	The antioxidant activity of AOS was evaluated by lipid oxidation inhibition, radical scavenging activity, and ferrous ion chelating activity. AOS were able to completely inhibit lipid oxidation in emulsions. AOS showed excellent radical scavenging activity towards ABTS, hydroxyl, and superoxide radicals, but had no ferrous ion chelating activity. The radical scavenging activity is suggested to originate mainly from the presence of the conjugated alkene acid structure formed during enzymatic depolymerization. According to the resonance hybrid theory, the parent radicals of AOS are delocalized through allylic rearrangement, and as a consequence, the reactive intermediates are stabilized.	[102]
Porphyridium cruentum poly-saccharides were degraded using hermetical microwave resulting in different molecules with 2918 to 256.2, 60.66 and 6.55 kDa.	The antioxidant properties of the degradation products were evaluated by determining the scavenging ability of free radicals, inhibitory effects on lipid peroxidation in liver homogenates and haemolysis of mouse erythrocytes. The low-molecular-weight fragments after degradation exerted an inhibitory effect on oxidative damage. The 6.55-kDa fragment had stronger antioxidant activity than the 60.66 and 256-kDa fragments.	[44]
Glucuronomannan oligosaccharides (GS) were firstly obtained by H_2O_2 degradation of fucoidan that was extracted from the brown alga *Sargassum thunbergii*. Sulfated glucurono-mannan oligosaccharides (SGS) were obtained by sulfation of GS.	Antioxidant activities (hydroxyl radical scavenging activity, superoxide radical scavenging activity, reducing power and DPPH radical scavenging activity) of Gs and SGs were determined. The higher the degree of polymerization the greater the antioxidant strength, except for the hydroxyl radical scavenging activity. On the other hand, the higher the sulfate content, the lower the reducing power and the DPPH radical scavenging activity. Opposite results were found for the superoxide radical scavenging activity. Compared with fucoidan, most GS and SGS showed higher antioxidant activity.	[103]
Wheat bran insoluble dietary fiber and an oxalic acid solution were mixed and boiled for 5 h. Soluble feruloylated oligosaccharides (FEOS) were separated on a Sephadex LH-20 gel filtration column eluted with 25% (v/v) ethanol/water.	Structural characterization demonstrated that the four fractions of FEOS contained esterified ferulic acid, arabinose and xylose linked by beta (1-4) glycosyl glycosidic bonds. FEOS revealed a concentration-dependent antioxidant activity as free radical scavengers (DPPH and hydroxyl), in reduction ability and metal ion chelation. FEOS-2 showed the best antioxidant potential. The antioxidant capacity was not only influenced by the amount of esterified ferulic acid but might be related to physical and chemical properties, such as particle size, solubility and viscosity.	[104]
A mulberry polysaccharide was firstly extracted in a water bath at 80 °C for 4 h and precipitated with ethanol. The crude mulberry poly-saccharide solution was then incubated with β-mannanase. The resulting oligosaccharides were purified by DEAE-52 cellulose and Sephadex G-100 column.	One of the oligossacharides, EMOS-1a, consisted of galactose units with an average molecular weight of 987 Da. The antioxidant activity of EMOS-1a, evaluated by as the DPPH and ABTS radical scavenging activities and ferric reducing antioxidant power (FRAP), correlated positively with its concentration.	[105]
A *Gracilaria* (red algae) crude polysaccharide was hydrolysed with agarase into neoagaro oligo-saccharides (NAOS) with different degrees of polymerization.	NAOS exhibited antioxidant capacity as determined by different methods, DPPH, ABTS, superoxide and hydroxyl radical scavenging activities and FRAP. The analysis showed that the degrees of polymerization can affect the antioxidant capacity of NAOSs.	[76]
Enzymatic Synthesis		
Daidzein was converted into 7-*O*-[6-*O*-(4-*O*-(β-D-xylopyranosyl))-β-D-xylopyranosyl]-β-D-glucopyran-oside by means of two enzymatic steps.	Cultured cells of *Catharanthus roseus* were used to convert daidzein into its 4'-*O*-β-glucoside, 7-*O*-β-glucoside, and 7-*O*-β-primeveroside. The latter was xylosylated by a *Aspergillus* sp. β-xylosidase to daidzein trisaccharide, 7-*O*-[6-*O*-(4-*O*-(β-D-xylopyranosyl))-β-D-xylopyranosyl]-β-D-glucopyranoside. The β-glucosides and β-xylooligosaccharide of daidzein exerted DPPH free-radical and superoxide radical scavenging activities.	[106]

The 2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH scavenging activity) is the most commonly used method for the study of oligosaccharides, probably because of its simplicity, speed and low cost compared to other methods [80]. Other recently used methods for evaluating the antioxidant capacity of oligosaccharides include the 2,2′-azino-bis (3-ethylbenzothiazoline sulfonic acid) radicals scavenging activity (ABTS scavenging activity), hydroxyl radical scavenging activity, ferric reducing antioxidant power (FRAP) superoxide anion radical scavenging, reducing power, and ferrous ion chelating activity. The principles, advantages and disadvantages of these methods can be accessed in specialized reviews [2, 107, 108].

3.2. *In vivo* Studies

The evaluation of the antioxidant capacity of different molecules can be performed following their effects on the redox state of different biological fluids and tissues, such as plasma, erythrocytes, urine and cerebrospinal fluids of humans and experimental animals. The eventual antioxidants to be tested are administered to the animals which are euthanized after given periods of time. Samples of blood, tissues or organs are used to

assess oxidative stress marker levels or eventual molecular or cell damages [81]. Endogenous enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) and non-enzymatic molecules such as reduced glutathione (GSH) act to reduce the levels of ROS [109, 110]. When ROS levels increase due to the increase in oxidants or deficiencies in cellular antioxidants, an oxidation-reduction imbalance occurs defined as oxidative stress, favoring an oxidative cellular environment [111, 112]. As a result of oxidative stress, for example, hepatic damage may occur, which impairs the liver functions and leads to many complications, such as immediate metabolic dysfunctions. These lesions initiate cell necrosis, fibrosis, lipid peroxidation, reduction in glutathione levels, and increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma [113].

End products of lipid peroxidation, such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal [114] can also be used as biomarkers of oxidative stress. The widespread use of MDA as a biomarker of lipid peroxidation is due to its reaction with DNA forming MDA-DNA adducts [115] and its easy reaction with thiobarbituric acid (TBAR) [116].

Total antioxidant capacity (T-AOC) of plasma is an important biomarker of oxidative stress, and useful in the evaluation of the antioxidant capacity of samples. T-AOC considers the synergistic role of all antioxidants (enzymatic and non-enzymatic) rather than the simple sum of individual antioxidants since it defines the synergistic effect between the various antioxidant compounds in the sample [117]. In addition to the enzymes already described above, the antioxidant system also comprises compounds such as uric acid, vitamin C, vitamin E, glutathione, bilirubin, α-lipoic acid, and carotenoids.

Table **2** presents recent studies in which different oligosaccharides had their antioxidant activities evaluated under *in vivo* conditions.

3.3. Structural Features of Oligosaccharides Determining Antioxidant Activity

Contaminants, especially phenolics and proteins, have been many times considered the reason for the overestimation of the antioxidant activities of crude extracts or partially purified oligosaccharides and polysaccharides. For this reason, it is absolutely necessary to use powerful methods of purification as well as strict criteria to confirm the homogeneity of the molecule

In the same way as the antioxidant action of polysaccharides [138-144], the antioxidant activities of oligosaccharides are affected by a myriad of reasons such as the method of depolymerization, the degree of polymerization, solubility, nature of the monosaccharide constituents, the glycosidic linkages of the side chains and the molecular weight [86, 96, 145-147]. A recent report shows that the transglycosylation products containing β-1,6-branched 3-O-β-D-gentiobiosyl-D-laminarioligosaccharides of laminaritriose reacted with a glucosidase which exhibited about 95% enhancement in the anti-oxidant activity compared to the untreated unbranched laminaritriose. This enhanced anti-oxidant activity was related to the production of a branched 3-O-β-D-gentiobiosyl residue [148]. In addition, the anti-oxidant activity of the laminarin containing more β-1,6 branches, isolated from *Eisenia bicyclis*, is stronger than that of the laminarin containing less β-1,6 branches, isolated from *Laminaria digitata* [149].

Furthermore, the reducing sugar content, the presence of phenolic groups such as ferulic acid, and the presence of uronic acid, among others, revealed to play an important role in the antioxidant properties [150, 151]. Still further, both polysaccharides and oligosaccharides have their antioxidant properties improved after chemical modifications, such as sulfation, carboxymethylation, phosphorylation, benzoylation, acetylation, among others [152]. After derivatization, both polysaccharides and oligosaccharides have their antioxidant activities increased [153-158].

An interesting improvement of antioxidant activity was obtained by chemical modification of a xanthan oligosaccharide. The antioxidant activity of the xanthan oligosaccharides is generally attributed to the reducing sugar and pyruvate acid contents [88]. The derivatives maleoyl xanthan oligosaccharides (XGOSMAs) and phthaloyl xanthan oligosaccharides (XGOSPAs), prepared by reacting xanthan oligosaccharides with maleic anhydride and phthalic anhydride, respectively, have similar substitution degrees, similar molecular weights, pyruvate acid and reducing sugar contents, but present higher antioxidant activity when compared to the non-reacted preparations [89]. The higher antioxidant properties of xanthan oligosaccharide derivatives have been attributed to the properties of these substituting groups [89].

Chitosan oligosaccharides (COS) are degradation products of chitosan, which is the N-deacetylated derivate of chitin, the second most abundant polymer in nature after cellulose. Chitin is found commercially in the waste products of the marine food processing industry, especially in those resulting from shrimp shell processing [159]. Compared to chitosan, COS have higher water solubility and lower viscosity, being absorbed through the intestine and excreted into the urine. The biological activities and therapeutic implications of COS were recently revised [156]. Several studies revealed that COS possess strong antioxidant and greater radical scavenging competency, showing potential biomedical applications [156-160]. The antioxidant activity of COS depends on their degree of deacetylation and molecular weights [156-160]. It was shown that 90% deacetylated medium molecular weight COS have the highest free radical scavenging activity against DPPH, hydroxyl, superoxide, and carbon-centered radicals [22]. The antioxidant properties are closely related to the amino and hydroxyl groups, which can react with unstable free radicals to form stable macromolecule radicals. Recently a commercial 85% deacetylated chitosan was degraded by a chitinase from *Coprinopsis cinerea* into several COS with degrees of polymerization ranging from 2 to 20 with a significant increase in the antioxidant activity, as evaluated by the DPPH-radical-scavenging activity method [161].

The antioxidant capacity of chitosan oligosaccharides can be modified by adding diverse organic groups to their structures [159-160]. The antioxidant properties of several modified chitosan oligosaccharides have been studied in detail [91, 92]. Besides increasing the spectrum of potentially useful molecules, such studies also contribute to a better understanding of the mechanisms underlying the antioxidant activity of these compounds. Good examples, among several others to be discussed below, are N-maleoyl chitosan oligosaccharide (NMCOS) and N-succinyl chitosan oligosaccharide (NSCOS). These derivatives were prepared from a chitosan oligosaccharide by acylation with maleic anhydride and succinic anhydride, respectively [91]. The antioxidant activities of these derivatives were evaluated by measuring superoxide anion $O_2^{\cdot-}$ and hydroxyl radical OH scavenging and by determining their reducing power. Results suggest that NMCOS possess stronger antioxidant activities, which may be related to the fact that the maleoyl moiety has a stronger electron-withdrawing effect than the succinyl moiety.

A N-furoyl chitosan oligosaccharide (NF-COS) was prepared *via* acylation of chitosan and chitosan oligosaccharide [92]. The NF-COS derivative exhibited higher antioxidant activities than the chitosan oligosaccharide, as determined by three methods: DPPH scavenging activity, reducing power and hydroxyl radical scavenging activity.

A gallate chitooligosaccharide derivative (gallate-COS) was obtained by covalently linking gallic acid to amino groups of chitooligosaccharide (COS) (Fig. **3**). The chemical structure of gallate-COS was identified by FT-IR, ^{1}H NMR and ^{13}C NMR [151]. COS and gallate-COS were found to be non-toxic and able to scavenge

Table 2. Antioxidant activities of oligosaccharides evaluated by *in vivo* methods.

Oligosaccharides and Experimental Systems	Most Important Observations and Conclusions	Refs.
Fructo-oligosaccharides (FOS)		
The effects of fructo-oligosac-charides (FOS) on fecal bifidobac-teria, lipid peroxidation index, indexes of nutritional status, and sustainability after withdrawal were studied in constipated nursing-home residents.	The supplementation of FOS increased the daily output of bifidobacteria, decreased plasma TBARS and cholesterol concentrations in constipated nursing-home elderly residents among other health beneficial effects. The effects remained at the end of the post-FOS period.	[118]
The effects of treatment with fructo-oligosaccharides (FOS) on intestinal mucositis induced by 5-fluorouracil (5-FU) were evaluated in mices. Oxidative stress was evaluated in fragments of ileum by measuring thiobarbituric acid-reactive species (TBARS), hydro-peroxide concentration and superoxide dismutase (SOD) plus catalase (CAT) activities.	No differences were observed with respect to lipid peroxidation and hydro-peroxide concentration in all investigated groups. However, the authors concluded that FOS supplementation in mucositis can improve cellular metabolism, preserving the catalase content and exerting antioxidant properties. FOS supplementation showed protective effects on the barrier function of the intestinal mucosa and may be an important adjunct in the prevention and treatment of mucositis.	[119]
The effects of *in ovo* and/or oral administration of the oligosaccharide from palm kernel cake on prenatal and post-hatched broiler chicks were evaluated.	Among other functional effects, the supplementation with oligosaccharides improved the total antioxidant capacities of serum and liver measured by both FRAP and ABTS methods. The analysis of antioxidant related genes (antioxidant enzymes), showed that the expression of catalase in the liver was significantly higher in the oligosaccharide palm kernel group than in the control group. However, no changes were observed in the expression of glutathione S-transferase-α and superoxide dismutase.	[120]
Alginate Oligosaccharides		
Oligosaccharide nanomedicine of alginate sodium (ONAS) was prepared with ampicillin at size <200 nm. ONAS was administered orally to patients with degenerative lumbar disease (DLD) osteoporosis. The purpose was to find out if ONAS can prevent some of the complications that follow the surgery that consists in posterior lumbar intervertebral fusion with cages (PLIFC).	After 1-month therapy, infection rates and side effects were lower in patients treated with ONAS than in those of the control group which received pluronic nanoparticles. The same occurred with the fusion rates (a measure for the success of the surgery). Compared to the control group, serum levels of miR-155, ALT, AST, and IL-1β were lower while SOD, GSH, and IL-1ra were higher in the ONAS group. The authors concluded that ONAS minimizes complications and improves the therapeutic effects after surgery in DLD by regulating serum miR-155 and by increasing the antioxidant activities by means of a down regulation of the serum levels of miR-155.	[121]
An alginate oligosaccharide (AOS) was prepared from alginate sodium of brown algae using alginate lyase. Four AOS with different degrees of poly-merization were produced and purified by size-exclusion chromatography. Only one AOS (DP5) had antitumor effects on osteosarcoma cells. Osteo-sarcoma patients were assigned into two groups: AOS (oral administration of 10-mg AOS-DP5 daily) and control groups (placebo).	AOS treatment resulted in increased serum levels of SOD, GSH, HDL-C, and reduced levels of interleukin-1 (IL-1) beta and IL-6. Treatment also diminished the plasma AST/ALT ratios and the plasma triglycerides, total cholesterol (TC), low-density lipoprotein cholesterol LDL-C, and malondialdehyde (MDA) levels. AOS reduced the osteosarcoma progression, which is associated with an improvement of the antioxidant and anti-inflammatory capacities of patients, suggesting its potential use as a drug for osteosarcoma therapy.	[122]
An alginate oligosaccharide obtained by alginic acid polysaccharide using alginate lyase, was used as a novel feed supplement in swine production. Growth performance, antioxidant capacity and intestinal digestion-absorption function in weaned pigs were evaluated and compared with a control group.	Superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA) and total antioxidant capacity (TAOC) were evaluated. A higher serum GSH content and CAT activity was observed in AOS-supplemented pigs than those in the control group. Also, AOS supplementation increased the serum T-AOC. No obvious differences in SOD activity and MDA content were observed between the two groups.	[123]
Mannan Oligosaccharides (MOS)		
MOS was used as food supplement and its effects on growth performance, antioxidant capacity, non-specific immunity and intestinal morphology of the Chinese mitten crab were evaluated.	Superoxide dismutase (SOD), total antioxidant capacity (T-AOC), malon-dialdehyde (MDA) and glutathione peroxidase (GSH-Px) in the hepatopancreas, intestine and serum were evaluated. The dietary MOS affected significantly the antioxidant capacity of the crabs.	[124]

Table 2 contd....

Oligosaccharides and Experimental Systems	Most Important Observations and Conclusions	Refs.
MOS was used as food supplement and its effects on growth performance, serum corticosterone level, antioxidant ability, meat quality and chemical composition of breast muscle was studied in broilers exposed to cyclic heat stress (HS).	MDA, reduced glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels were evaluated in breast muscle homogenate. The addition of MOS increased the GSH-Px activity and decreased the MDA content with the GSH-Px activity being similar to that in the control group. However, there were no differences in SOD activity and GSH content in the breast muscle among groups. MOS improved oxidative status in broilers under cyclic heat stress.	[125]
Pectin Oligosaccharides (POS)		
A study was conducted to investigate the effect of pectin oligosaccharides (POS) and zinc chelate (Zn-POS) on growth performance, zinc status, intestinal morphology and antioxidant status in broilers.	The oxidative status was inferred from the total antioxidant capacity (T-AOC), superoxide dismutase (SOD), copper-zinc superoxide dismutase (CuZn·SOD) and glutathione peroxidase (GSH-Px) activities besides the MDA contents in serum and liver. Supplementation of the diet with pectin oligosaccharides (POS) and zinc chelate (Zn-POS) had a powerful impact on the activities of enzymes and gene expression involved in the antioxidant status of broilers.	[126]
Chitosan Oligosaccharides (COS)		
The authors investigated the effects of dietary COS supplementation during late gestation on the antioxidant defence capacity of sows.	Maternal dietary COS supplementation increased plasma total SOD and caused a downtrend in plasma MDA. The mRNA expression of some antioxidant genes in the placenta were increased and pro-inflammatory cytokines were reduced by COS supplementation. No significant modifications were observed in the activities of placental total SOD and CAT. Maternal dietary supplementation with COS protected sows against oxidative stress by increasing plasma antioxidants and blocking the inflammatory response.	[127]
In this work, the authors investigated the effects of a *Forsythia suspensa* extract (FSE) and chitooligosaccharide (COS), alone or together, on per-formance and health status of weaned piglets.	The oxidative status was evaluated by determining the serum total antioxidant activity and the enzymes superoxide dismutase and glutathione peroxidase, and by quantifying the oxidative injury products 8-hydroxy-2′-deoxyguanosine (urine) and malondialdehyde (serum). The FSE or COS supplementation in post-weaning diets improves the performance and feed utilization, and decreases the severity of diarrhea. The beneficial effects of both FSE or COS may be attributed to the same underlying biological response mechanisms as assessed by reduced intestinal permeability, improved antioxidant capacity and enhanced immune function.	[35]
The authors evaluated the effects of chitosan oligosaccharides (COS) on coronary heart disease (CHD) patients.	Circulating antioxidant levels were higher in the COS group than in the control group. COS consumption increased the serum levels of SOD and GSH and reduced the levels of ALT and AST. The lipid profiles were improved in the COS group. In the same way, COS consumption increased the types and numbers of probiotic species of the intestinal flora.	[128]
The effects of chitooligosaccharides (COS) on growth, antioxidant capacity, non-specific immune response, and resistance to *Aeromonas hydrophila* in GIFT tilapia (*Oreochromis niloticus*) were evaluated.	COS supplementation improved the serum T-AOC (total antioxidant capacity) and decreased the serum MDA and catalase activities. No significant differences were observed in the serum SOD and GSH-Px activities among the dietary treatments. Results suggest that dietary COS supplementation could enhance the performance and the immune response of GIFT tilapia.	[129]
The authors investigated the possible anti-aging effect of COS using the mouse aging model induced by D-galactose (D-gal.)	The decreased activities of SOD, CAT, and GSH-Px caused by D-gal were gradually elevated to values comparable to those in the control group. The MDA level was attenuated by COS in a dose-dependent manner.	[130]
The objective of this study was to investigate the potential role of COS in doxorubicin (DOX)-induced cardio-toxicity, and the effects of COS on apoptosis and oxidative stress in rats and H9C2 cells.	Pretreatment with COS significantly reduced the high levels of MDA caused by DOX in the heart tissue. COS also reverted to normal levels the activities of CAT and SOD as well as the GSH level and the GSH/GSSG ratio.	[34]
The authors evaluated the effects of COS on NF-κB (nuclear factor kappa B) activation and MAPK (mitogen-ac-tivated protein kinases) phosphorylation in a rat model of retinal I/R injury induced by transiently raising the intra-ocular pressure.	COS diminished ROS production and retinal oxidative damage. It also inhibited NF-κB activation by decreasing IκB degradation and p65 expression. COS decreased phosphorylation of JNK and ERK, but increased the phosphorylation of p38.	[131]
The objective of this study was to analyse the antioxidant activities of chitooligosaccharides (COS) in a high-fat diet (HFD)-mouse model.	The administration of a high fat diet resulted in a reduction of the activities of superoxide dismutase, catalase and glutathione peroxidase in stomach, liver and serum of mice. The administration of COS, in association with the high fat diet, resulted in significant increases in the activities of the three enzymes. In conclusion, COS can restore the activities of the enzymes affected by the high fat diet.	[132]

Table 2 contd....

Oligosaccharides and Experimental Systems	Most Important Observations and Conclusions	Refs.
Feruloyl Oligosaccharides (FEOS) and Others		
The antioxidant activities of wheat bran feruloyl oligosaccharides (FEOS) were determined in rats by measuring the activities and mRNA expression levels of phase II detoxifying/antioxidant en-zymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and heme oxygenase-1 (HO-1) in rat organs, heart, liver, and kidney.	SOD, CAT, and GSH-Px in FEOS groups were significantly increased in heart, liver, and kidney when compared with the control group. The same occurred with the glutathione (GSH) contents in heart, liver, and kidney. FEOS up-regulated the mRNA expression levels of SOD, CAT, and HO-1 in the organs. The immunoblot analysis revealed increased nuclear factor-E2-related factor (Nrf2) protein expression levels in the organs and there were positive correlations between the mRNA expression of phase II detoxifying/antioxidant enzymes and the expressions of Nrf2 protein. The authors conclude that FEOS treatment could modulate the detoxifying/antioxidant enzymes *via* Nrf2 signaling.	[133]
In this work the effects of wheat bran feruloyl oligosaccharides (FEOS), as an antioxidant supplement for perform-ance, were investigated with respect to blood metabolite levels, antioxidant status and ruminal fermentation in lambs.	Compared to the control group, the serum catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities and glutathione (GSH) levels of lambs were significantly higher, while the serum total antioxidant capacity (T-AOC) slightly increased.	[78]
Wheat bran feruloyl oligosaccharides (FEOS) possess *in vitro* antioxidative potential. The aim of this study was to investigate the protective effect of FEOS against oxidative stress in rat plasma.	Compared to the control group, the antioxidant enzyme activities (superoxide dismutase, catalase and glutathione peroxidase) were higher in plasma from rats fed with FEOS and oxidised glutathione and malondialdehyde levels were lower. After ingestion of FEOS, the plasma of rats was more resistant to AAPH-induced haemolysis compared to the control group. These results suggest that FEOS enhance the level of the antioxidant activity in rat plasma *in vivo*.	[134]
The mechanisms by which wheat bran feruloyl oligosaccharides (FEOS) pro-tect against 2,2′-azobis(2-methylpro-pionamidine) dihydrochloride (AAPH) induced oxidant injury were in-vestigated in rats.	The FEOS treated group had the highest activities and mRNA expression levels of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). The activities of SOD, CAT, and GPx positively correlated with the mRNA and protein expression levels of Nrf2. The FEOS group increased the mRNA expression level of Nrf2 and down regulated the expression level of kelch-like ECH-associated protein-1 (Keap1), demonstrating that FOs could cause a dissociation of the Nrf2/Keap1 complex. The upstream signaling of Nrf2, gene and protein expression levels of p38 mitogen-activated protein kinases (MAPK) and phosphatidylinositol-3-kinase (PI3K) were up-regulated by FOs. Pretreatment of FOsH increased the mRNA and protein expression levels of masculoaponeurotic fibrosarcoma K (MafK) but not MafG and MafF.	[135]
The objective of this study was to evaluate the protective effect of a com-bination supplementation of fructo- and xylooligosaccharides (FOS + XOS) during perinatal period aiming to mitigate acrylamide-induced oxidative stress and neurotoxicity in mothers (rats) and young pups.	Acrylamide exposure caused a significant reduction in the maternal gestational/lactational body weight and preweaning body weight as well as behavioral alterations among male offspring. The combination supplement of FOS + XOS had no significant effect on these modifications. However, significantly diminished antioxidant enzyme (SOD and CAT) activities in the maternal and offspring brain were restored in rats given FOS+XOS supplementation. The prebiotic supplementation normalized the elevated nitric oxide levels in the cerebellum of the offspring born to ACR exposed rats. Furthermore, prebiotics restored the activity of acetylcholinesterase (AChE) and improved the levels of dopamine (DA) in the maternal cortex. The protective effect of prebiotic supplementation was also discernible in the mitochondrial fraction of maternal brain regions. These findings suggest that prebiotic supplementation during pregnancy may be useful in attenuating the perinatal toxic effects associated with neurotoxin exposure.	[136]
Sulfate oligosaccharides from green algae *Ulva lactuca* (ULO) and *Enteromorpha prolifera* (EPO) were used for investigating anti-aging effects and the underlying mechanism in SAMP8 mice.	The oligosaccharides enhanced the glutathione, superoxide dismutase, catalase, and telomerase levels and the total antioxidant capacity, and decreased the levels of malondialdehyde and advanced glycation end products. After ULO and EPO treatment, the levels of inflammatory factors, including IFN-γ, TNF-α, and IL-6, decreased; the BDNF and ChAT levels increased; and hippocampal neurons were protected. Down-regulation of the p53 and FOXO1 genes and upregulation of the Sirt1 gene indicate that ULO and EPO have potential therapeutic effects in the prevention of aging in SAMP8 mice. By 16S rRNA gene high-throughput sequencing, the abundance of *Desulfo-vibrio* was found to be markedly different in mice treated with ULO and EPO. The abundances of *Verrucomicrobiaceae*, *Odoribacteraceae*, *Mogibacteriaceae*, *Planococcaceae*, and *Coriobacteriaceae* correlated positively with the age-related indicators.	[137]

cellular radicals in RAW264.7 cells. Both COS and gallate-COS inhibit oxidative damage to lipids, proteins and DNA in RAW264.7 cells, decrease the activation and expression of NF-κB and increase the level of intracellular antioxidant enzymes (SOD and GSH) in oxidative stress-induced RAW264.7 cells. Collectively, gallate-COS could be used as scavengers to control free radicals that lead to damage to cellular systems.

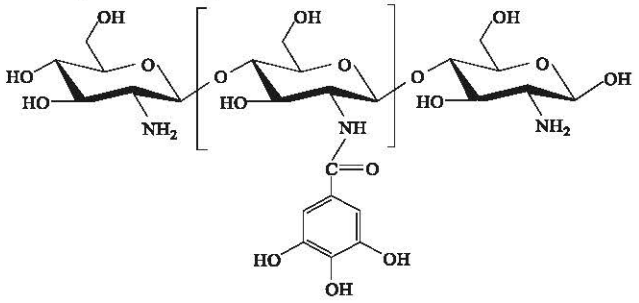

Fig. (3). Galloyl-chitooligossacharide structure.

Xylooligosaccharides (XOS) are sugar oligomers formed by xylose units, which appear naturally in fruits, vegetables, milk, and honey. For large scale, XOS are obtained from hydrolysis reactions involving arabinoxylans derived from lignocellulosic materials, or cereal and millet brans [60, 92, 93, 95, 162]. The antioxidant activity of xylo-oligosaccharides is generally attributed to the presence of ester-linked hydroxycinnamic acid derivatives, such as ferulic acid, coumaric caffeic and syringic acid residues on the xylan chain [78, 96, 102, 131, 132, 133, 163] (Fig. 4). Members of feruloylated oligo-saccharides may differ from each other in terms of composition and number of glycosylated monosaccharides, the species of sugar residues linked to ferulic acid and the linking position, the contents of ferulic acid, and whether they contain di-, tri-, tetra-ferulic acid or *p*-coumaric acid.

Feruloylated oligosaccharides owe their nutritional functions to both ferulic acid and oligosaccharides. They are stable under low pH and high temperature. As excellent functional ingredients, feruloylated oligosaccharides have a wide range of applications in the food industry [81, 100].

4. PERSPECTIVES

In recent years, the concept of the linear economy has been replaced by the concept of a circular economy, since the linear model is based primarily on the use of non-renewable fossil resources. The concept of a circular economy endorses the approach to recycling, reuse, closing the product life cycle [164]. Within this context, the use of agro-industrial waste, such as lignocellulosic materials to obtain xylooligosaccharides and xylo-oligosaccharide derivatives, and marine food processing industry waste, including shrimp shell, to obtain chitooligosaccharides and chitooligosaccharide derivatives, represent applications of the circular economy concept, linked to the bio-based economy [165-172].

Green technology could also be incorporated more frequently in the obtainment of antioxidant oligosaccharides. Although the extraction process using water is the most economic one, it is not selective and several interferents are coextracted. Besides this, high temperatures are generally required to improve efficiency, which can lead to degradation of thermolabile oligosaccharides. Additionally, other advanced techniques can be routinely used in the future, including microwave-assisted extraction, pressurized liquid extraction, supercritical fluid extraction and subcritical water treatment. The latter has already been used as an effective method for the obtainment of oligosaccharides from passion fruit peel [173] and from *Pleurotus eryngii* [174]. Other green technology methods such as the use of ionic liquids for the extraction and fractionation, microwave-assisted extraction, ultrasound-assisted extraction, pressurized liquid extraction, supercritical fluid extraction, and enzyme-assisted extraction, have emerged during the last decades [175] and will certainly be useful in the obtainment of antioxidant oligosaccharides.

SUMMARIZING CONCLUSION

The above considerations gain substantial significance and importance if one considers the quite numerous and generally consistent reports that were detailed in this literature review regarding the antioxidant properties of non-digestible oligosaccharides. The production on a large scale of antioxidant oligosaccharides is still a challenge for food science and technology. However, several methods have been recently developed, modified, and adapted to optimize the production of different oligosaccharides. The food industry needs more efficient, simple, and less expensive processes for their application on a large scale.

In conclusion, the consumption of antioxidant oligosaccharides may be beneficial to human and animal health. The main benefits can be expected in the case of diseases that modify substantially the redox status of patients, such as diabetes, cardiovascular disease, colon cancer, obesity, abnormal lipid metabolism and chronic inflammatory diseases such as rheumatoid arthritis. However, experimental approaches where different oligosaccharides and oligosaccharide derivatives can be evaluated in parallel are

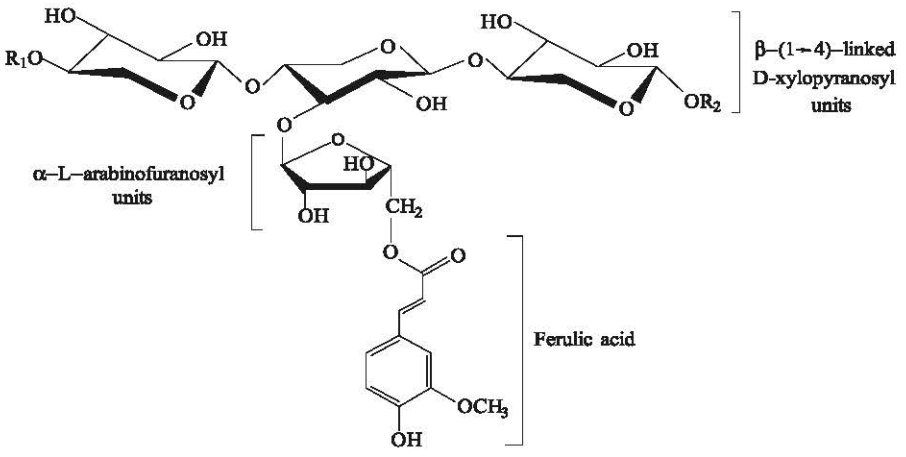

Fig. (4). Feruloyl xylooligosaccharide structure.

necessary to elucidate the mechanisms of action and the real benefits of consuming these compounds in the control of oxidative stress.

CONSENT FOR PUBLICATION

Hereby, as the corresponding author and in the name of all co-authors, I express my consent for publication of the article "An overview of structural aspects and health beneficial effects of antioxidant oligosaccharides" in Current Pharmaceutical Design. Rosane Marina Peralta.

FUNDING

This work was funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil; grants numbers 40898/2016-5 and 307944/2015-8).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

T.F. Vieira thanks Fundação Araucária-Paraná-Brazil for fellowship. R.C.G. Corrêa's work is supported by the Cesumar Institute of Science, Technology and Innovation (ICETI). R.A. Peralta, R.F. Peralta-Muniz-Moreira, A. Bracht and R.M. Peralta are CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) grant recipients.

REFERENCES

[1] Haminiuk CWI, Maciel GM, Plata-Oviedo MSV, *et al.* Phenolic compounds in fruits: an overview. Int J Food Sci Technol 2012; 47: 2023-44. http://dx.doi.org/10.1111/j.1365-2621.2012.03067.x

[2] Carocho M, Ferreira ICFR. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol 2013; 51: 15-25. http://dx.doi.org/10.1016/j.fct.2012.09.021 PMID: 23017782

[3] Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem 2015; 97: 55-74. http://dx.doi.org/10.1016/j.ejmech.2015.04.040 PMID: 25942353

[4] Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects-A review. J Funct Foods 2015; 18: 820-97. http://dx.doi.org/10.1016/j.jff.2015.06.018

[5] Martins N, Barros L, Ferreira ICFR. *In vivo* antioxidant activity of phenolic compounds: Facts and gaps. Trends Food Sci Technol 2016; 48: 1-12. http://dx.doi.org/10.1016/j.tifs.2015.11.008

[6] Carocho M, Morales P, Ferreira ICFR. Antioxidants: Reviewing the chemistry, food applications, legislation and role as preservatives. Trends Food Sci Technol 2018; 71: 107-20. http://dx.doi.org/10.1016/j.tifs.2017.11.008

[7] Corrêa RCG, Peralta RM, Haminiuk CWI, Maciel GM, Bracht A, Ferreira ICFR. New phytochemicals as potential human anti-aging compounds: Reality, promise, and challenges. Crit Rev Food Sci Nutr 2018; 58(6): 942-57. http://dx.doi.org/10.1080/10408398.2016.1233860 PMID: 27623718

[8] Neha K, Haider MR, Pathak A, Yar MS. Medicinal prospects of antioxidants: A review. Eur J Med Chem 2019; 178: 687-704. http://dx.doi.org/10.1016/j.ejmech.2019.06.010 PMID: 31228811

[9] Harris IS, Treloar AE, Inoue S, *et al.* Glutathione and thioredoxin antioxidant pathways synergize to drive cancer initiation and progression. Cancer Cell 2015; 27(2): 211-22. http://dx.doi.org/10.1016/j.ccell.2014.11.019 PMID: 25620030

[10] Kashyap C, Mazumder LJ, Rohman SS, *et al.* Re-visiting the antioxidant activity of Se-and Te-carbohydrates: a theoretical study. ChemistrySelect 2019; 4: 1470-5. http://dx.doi.org/10.1002/slct.201803814

[11] Arulselvan P, Fard MT, Tan WS, *et al.* Role of antioxidants and natural products in inflammation. Oxid Med Cell Longev 2016; 2016: 5276130. http://dx.doi.org/10.1155/2016/5276130 PMID: 27803762

[12] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 4th ed. Oxford: Oxford University Press 2007.

[13] Granato D, Shahidi F, Wrolstad R, *et al.* Antioxidant activity, total phenolics and flavonoids contents: Should we ban *in vitro* screening methods? Food Chem 2018; 264: 471-5. http://dx.doi.org/10.1016/j.foodchem.2018.04.012 PMID: 29853403

[14] Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. Rsc Adv 2015; 5: 27986-8006. http://dx.doi.org/10.1039/C4RA13315C

[15] Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alexandria J Med 2018; 54: 287-93. http://dx.doi.org/10.1016/j.ajme.2017.09.001

[16] Oroian M, Escriche I. Antioxidants: Characterization, natural sources, extraction and analysis. Food Res Int 2015; 74: 10-36. http://dx.doi.org/10.1016/j.foodres.2015.04.018 PMID: 28411973

[17] Kungel PTAN, Correa VG, Corrêa RCG, *et al.* Antioxidant and antimicrobial activities of a purified polysaccharide from yerba mate (*Ilex paraguariensis*). Int J Biol Macromol 2018; 114: 1161-7. http://dx.doi.org/10.1016/j.ijbiomac.2018.04.020 PMID: 29627472

[18] Ferreira ICFR, Heleno SA, Reis FS, *et al.* Chemical features of *Ganoderma* polysaccharides with antioxidant, antitumor and antimicrobial activities. Phytochemistry 2015; 114: 38-55. http://dx.doi.org/10.1016/j.phytochem.2014.10.011 PMID: 25457487

[19] Zhang J, Wen C, Zhang H, Duan Y. Review of isolation, structural properties, chain conformation, and bioactivities of psyllium polysaccharides. Int J Biol Macromol 2019; 139: 409-20. http://dx.doi.org/10.1016/j.ijbiomac.2019.08.014 PMID: 31381918

[20] Gurpilhares DB, Cinelli LP, Simas NK, Pessoa A Jr, Sette LD. Marine prebiotics: Polysaccharides and oligosaccharides obtained by using microbial enzymes. Food Chem 2019; 280: 175-86. http://dx.doi.org/10.1016/j.foodchem.2018.12.023 PMID: 30642484

[21] Naveed M, Phil L, Sohail M, *et al.* Chitosan oligosaccharide (COS): An overview. Int J Biol Macromol 2019; 129: 827-43. http://dx.doi.org/10.1016/j.ijbiomac.2019.01.192 PMID: 30708011

[22] Yuan X, Zheng J, Jiao S, *et al.* A review on the preparation of chitosan oligosaccharides and application to human health, animal husbandry and agricultural production. Carbohydr Polym 2019; 220: 60-70. http://dx.doi.org/10.1016/j.carbpol.2019.05.050 PMID: 31196551

[23] Patel S, Goyal A. Functional oligosaccharides: production, properties and applications. World J Microbiol Biotechnol 2010; 27: 1119-28. http://dx.doi.org/10.1007/s11274-010-0558-5

[24] Van den Ende W. Multifunctional fructans and raffinose family oligosaccharides. Front Plant Sci 2013; 4: 247-57. PMID: 23882273

[25] Roberfroid M, Gibson GR, Hoyles L, *et al.* Prebiotic effects: metabolic and health benefits. Br J Nutr 2010; 104(S2)(Suppl. 2): S1-S63. http://dx.doi.org/10.1017/S0007114510003363 PMID: 20920376

[26] Jovanovic-Malinovska R, Kuzmanova S, Winkelhausen E. Oligosaccharide profile in fruits and vegetables as sources of prebiotics and functional foods. Int J Food Prop 2014; 17: 949-65. http://dx.doi.org/10.1080/10942912.2012.680221

[27] Zhao C, Wu Y, Liu X, *et al.* Functional properties, structural studies and chemo-enzymatic synthesis of oligosaccharides. Trends Food Sci Technol 2017; 66: 135-45. http://dx.doi.org/10.1016/j.tifs.2017.06.008

[28] Oliveira DL, Andree Wilbey R, Grandison AS, *et al.* Milk oligosaccharides: a review. Int J Dairy Technol 2015; 68: 305-21. http://dx.doi.org/10.1111/1471-0307.12209

[29] Boehm G, Stahl B. Oligosaccharides from milk. J Nutr 2007; 137(3)(Suppl. 2): 847S-9. http://dx.doi.org/10.1093/jn/137.3.847S PMID: 17311985

[30] Haslam SM, North SJ, Dell A. Mass spectrometric analysis of N- and O-glycosylation of tissues and cells. Curr Opin Struct Biol 2006; 16(5): 584-91.
http://dx.doi.org/10.1016/j.sbi.2006.08.006 PMID: 16938453

[31] Ashwini A, Ramya HN, Ramkumar C, *et al.* Reactive mechanism and the applications of bioactive prebiotics for human health: Review. J Microbiol Methods 2019; 159: 128-37.
http://dx.doi.org/10.1016/j.mimet.2019.02.019 PMID: 30826441

[32] Mano MCR, Neri-Numa IA, da Silva JB, Paulino BN, Pessoa MG, Pastore GM. Oligosaccharide biotechnology: an approach of prebiotic revolution on the industry. Appl Microbiol Biotechnol 2018; 102(1): 17-37.
http://dx.doi.org/10.1007/s00253-017-8564-2 PMID: 29032473

[33] Xiao X, Wen JY, Wang YY, *et al.* NMR and ESI-MS spectrometry characterization of autohydrolysis xylo-oligosaccharides separated by gel permeation chromatography. Carbohydr Polym 2018; 195: 303-10.
http://dx.doi.org/10.1016/j.carbpol.2018.04.088 PMID: 29804981

[34] Zhang Y, Ahmad KA. Chitosan oligosaccharides prevent doxorubicin-induced oxidative stress and cardiac apoptosis through activating p38 and JNK MAPK mediated Nrf2/ ARE pathway Chem - Biol Interac 2019; 305: 54-65.

[35] Zhao P, Piao X, Zeng Z, Li P, Xu X, Wang H. Effect of *Forsythia suspensa* extract and chito-oligosaccharide alone or in combination on performance, intestinal barrier function, antioxidant capacity and immune characteristics of weaned piglets. Anim Sci J 2017; 88(6): 854-62.
http://dx.doi.org/10.1111/asj.12656 PMID: 27758020

[36] Wang Y, Guo Q, Goff HD, *et al.* Oligosaccharides: structure, function and application.Encyclopedia of Food Chemistry. Amsterdam: Elsevier 2019; 202-7.
http://dx.doi.org/10.1016/B978-0-08-100596-5.21585-0

[37] Mussatto SI, Mancilha IM. Non-digestible oligosaccharides: a review. Carbohydr Polym 2007; 68: 587-97.
http://dx.doi.org/10.1016/j.carbpol.2006.12.011

[38] Arruda HS, Pereira GA, Almeida MEF, *et al.* Current knowledge and future perspectives of oligosaccharides research. Frontiers in Natural Product Chemistry. Sharjah: Bentham Science 2017; 91-175.

[39] Moura FA, Macagnan FT, Silva LP. Oligosaccharide production by hydrolysis of polysaccharides: a review. Int J Food Sci Technol 2015; 50: 275-81.
http://dx.doi.org/10.1111/ijfs.12681

[40] Nobre C, Cerqueira MÂ, Rodrigues LR, *et al.* Production and extraction of polysaccharides and oligosaccharides and their use as new food additives.Industrial Biorefineries and White Biotechnology. Amsterdam: Elsevier 2015; pp. 653-79.
http://dx.doi.org/10.1016/B978-0-444-63453-5.00021-5

[41] Lin X, Chen J, Xiao G, *et al.* Extraction, molecular weight distribution, and antioxidant activity of oligosaccharides from longan (*Dimocarpus Longan* Lour.) pulp. Food Sci Biotechnol 2016; 25(3): 701-6.
http://dx.doi.org/10.1007/s10068-016-0122-7 PMID: 30263326

[42] Nguyen TH, Haltrich D. Microbial production of prebiotic oligosaccharides.Microbial production of food ingredients, enzymes and nutraceuticals. Indiana: Woodhead Publishing Limited 2013; pp. 494-530.
http://dx.doi.org/10.1533/9780857093547.2.494

[43] Wu S, Cai R, Sun Y. Degradation of curdlan using hydrogen peroxide. Food Chem 2012; 135(4): 2436-8.
http://dx.doi.org/10.1016/j.foodchem.2012.07.077 PMID: 22980825

[44] Sun L, Wang C, Shi Q, Ma C. Preparation of different molecular weight polysaccharides from *Porphyridium cruentum* and their antioxidant activities. Int J Biol Macromol 2009; 45(1): 42-7.
http://dx.doi.org/10.1016/j.ijbiomac.2009.03.013 PMID: 19447258

[45] Smoot JT, Demchenko AV. Oligosaccharide synthesis: from conventional methods to modern expeditious strategies. Adv Carbohydr Chem Biochem 2009; 62: 161-250.
http://dx.doi.org/10.1016/S0065-2318(09)00005-5 PMID: 19501706

[46] Barreteau H, Delattre C, Michaud P. Production of oligosaccharides as promising new food additive generation. Food Technol Biotechnol 2006; 44: 323-33.

[47] Yip VL, Withers SG. Breakdown of oligosaccharides by the process of elimination. Curr Opin Chem Biol 2006; 10(2): 147-55.
http://dx.doi.org/10.1016/j.cbpa.2006.02.005 PMID: 16495121

[48] Abdul Manas NH, Md Illias R, Mahadi NM. Strategy in manipulating transglycosylation activity of glycosyl hydrolase for oligosaccharide production. Crit Rev Biotechnol 2018; 38(2): 272-93.
http://dx.doi.org/10.1080/07388551.2017.1339664 PMID: 28683572

[49] Mangas-Sánchez J, Adlercreutz P. Enzymatic preparation of oligosaccharides by transglycosylation: A comparative study of glucosidases. J Mol Catal, B Enzym 2015; 122: 51-5.
http://dx.doi.org/10.1016/j.molcatb.2015.08.014

[50] Bhatia Y, Mishra S, Bisaria VS. Microbial β-glucosidases: cloning, properties, and applications. Crit Rev Biotechnol 2002; 22(4): 375-407.
http://dx.doi.org/10.1080/07388550290789568 PMID: 12487426

[51] Withers SG. Mechanisms of glycosyl transferases and hydrolases. Carbohydr Polym 2001; 44: 325-37.
http://dx.doi.org/10.1016/S0144-8617(00)00249-6

[52] Bemiller JN. Oligosaccharides. Carbohydrate Chemistry for Food Scientists. Indiana: Woodhead Publishing and AACC International Press 2019; 49-74.
http://dx.doi.org/10.1016/B978-0-12-812069-9.00003-0

[53] Hsu HC, Liew CY, Huang SP, Tsai ST, Ni CK. Simple method for de novo structural determination of underivatised glucose oligosaccharides. Sci Rep 2018; 8(1): 5562.
http://dx.doi.org/10.1038/s41598-018-23903-4 PMID: 29615745

[54] Gómez B, Míguez B, Yáñez R, *et al.* Extraction of oligosaccharides with prebiotic properties from agro-industrial wastes.Water extraction of bioactive compounds: From plants to drug development. Amsterdam: Elsevier 2018; 131-61.

[55] Sanz ML, Ruiz-Matute AI, Corzo N, *et al.* Analysis of prebiotic oligosaccharides. Prebiotics and Probiotics Science and Technology. New York: Springer 2009; 465-534.
http://dx.doi.org/10.1007/978-0-387-79058-9_13

[56] Bai W, Fang X, Zhao W, *et al.* Determination of oligosaccharides and monosaccharides in Hakka rice wine by pre-column derivation high-performance liquid chromatography. Yao Wu Shi Pin Fen Xi 2015; 3: 1-7.

[57] Lane JA, Hickey RM. Analysis of bioactive food-sourced oligosaccharides by high-performance liquid chromatography.food oligosaccharides: production, analysis and bioactivity. New Jersey: John Wiley & Son 2014; 399-420.
http://dx.doi.org/10.1002/9781118817360.ch21

[58] Reiffová K. Analysis of food bioactive oligosaccharides by thin-layer chromatography. Food oligosaccharides: production, analysis and bioactivity. New Jersey: John Wiley & Son 2014; 350-69.
http://dx.doi.org/10.1002/9781118817360.ch19

[59] Yan J, Ding J, Liang X. Chromatographic methods for analysis of oligosaccharides in human milk. Anal Methods 2017; 9: 1071-7.
http://dx.doi.org/10.1039/C6AY02982E

[60] Kaur R, Uppal SK, Sharma P. Production of xylooligosaccharides from sugarcane bagasse and evaluation of their prebiotic potency *in vitro*. Waste Biomass Valoriz 2018; 10: 2627-35.
http://dx.doi.org/10.1007/s12649-018-0266-1

[61] Herrero M, Cifuentes A, Ibáñez E, Dolores M. Advanced analysis of carbohydrates in foods.methods of analysis of food components and additives. Boca Raton: CRC Press 2012; 135-64.

[62] Soria AC, Rodríguez-Sanchéz S, Sanz J, *et al.* Gas chromatographic analysis of food bioactive-oligosaccharides.food oligosaccharides: production, analysis and bioactivity. New Jersey: John Wiley & Son 2014; 370-98.
http://dx.doi.org/10.1002/9781118817360.ch20

[63] Sabater C, Ferreira-Lazarte A, Montilla A, Corzo N. Enzymatic production and characterization of pectic oligosaccharides derived from citrus and apple pectins: A GC-MS study using random forests and association rule learning. J Agric Food Chem 2019; 67(26): 7435-47.
http://dx.doi.org/10.1021/acs.jafc.9b00930 PMID: 31244205

[64] Raessler M. Sample preparation and current applications of liquid chromatography for the determination of non-structural carbohydrates in plants. Trends Analyt Chem 2011; 2011(30): 1833-43.
http://dx.doi.org/10.1016/j.trac.2011.06.013

[65] Zang H, Xie S, Zhu B, *et al.* Mannan oligosaccharides trigger multiple defence responses in rice and tobacco as a novel danger-associated molecular pattern. Mol Plant Pathol 2019; 20(8): 1067-79.
http://dx.doi.org/10.1111/mpp.12811 PMID: 31094073

[66] Pu J, Zhao X, Wang Q, Wang Y, Zhou H. Development and validation of a HPLC method for determination of degree of polymerization of xylo-oligosaccharides. Food Chem 2016; 213: 654-9. http://dx.doi.org/10.1016/j.foodchem.2016.07.014 PMID: 27451231

[67] Cao L, Tian H, Wu M, Zhang H, Zhou P, Huang Q. Determination of curdlan oligosaccharides with high-performance anion exchange chromatography with pulsed amperometric detection. J Anal Methods Chem 2018; 2018: 3980814. http://dx.doi.org/10.1155/2018/3980814 PMID: 31049244

[68] Szilágyi TG, Vecseri BH, Kiss Z, Hajba L, Guttman A. Analysis of the oligosaccharide composition in wort samples by capillary electrophoresis with laser induced fluorescence detection. Food Chem 2018; 256: 129-32. http://dx.doi.org/10.1016/j.foodchem.2018.02.106 PMID: 29606428

[69] Mechelke M, Herlet J, Benz JP, *et al.* HPAEC-PAD for oligosaccharide analysis-novel insights into analyte sensitivity and response stability. Anal Bioanal Chem 2017; 409(30): 7169-81. http://dx.doi.org/10.1007/s00216-017-0678-y PMID: 29026979

[70] Duan W, Ji W, Wei Y, *et al.* Separation and purification of fructo-oligosaccharide by high-speed counter-current chromatography coupled with precolumn derivatization. Molecules 2018; 23(2): 381-9. http://dx.doi.org/10.3390/molecules23020381 PMID: 29439422

[71] Pu J, Zhao X, Wang Q, *et al.* Structural characterization of xylo-oligosaccharides from corncob residues. J Carbohydr Chem 2016; 35: 344-54. http://dx.doi.org/10.1080/07328303.2016.1239107

[72] Valls C, Pastor FIJ, Vidal T, *et al.* Antioxidant activity of xylooligosaccharides produced from glucuronoxylan by Xyn10A and Xyn30D xylanases and eucalyptus autohydrolysates. Carbohydr Polym 2018; 194: 43-50. http://dx.doi.org/10.1016/j.carbpol.2018.04.028 PMID: 29801857

[73] Zou P, Yuan S, Yang X, *et al.* Structural characterization and antitumor effects of chitosan oligosaccharides against orthotopic liver tumor *via* NF-κB signaling pathway. J Funct Foods 2019; 57: 157-65. http://dx.doi.org/10.1016/j.jff.2019.04.002

[74] Kailemia MJ, Ruhaak LR, Lebrilla CB, Amster IJ. Oligosaccharide analysis by mass spectrometry: a review of recent developments. Anal Chem 2014; 86(1): 196-212. http://dx.doi.org/10.1021/ac403969n PMID: 24313268

[75] Hernández-Hernández O, Roepstorff P. Mass spectrometric analysis of food bioactive oligosaccharides. food oligosaccharides: production, analysis and bioactivity. New Jersey: John Wiley & Son 2014; 439-56. http://dx.doi.org/10.1002/9781118817360.ch23

[76] Zhang YH, Song XN, Lin Y, *et al.* Antioxidant capacity and prebiotic effects of *Gracilaria* neoagaro oligosaccharides prepared by agarase hydrolysis. Int J Biol Macromol 2019; 137: 177-86. http://dx.doi.org/10.1016/j.ijbiomac.2019.06.207 PMID: 31255619

[77] Xiong Y, Wang Y, Li M, *et al.* HPAEC-PAD and Q-TOF-MS/MS analysis reveal a novel mode of action of endo-β-1,3(4)-d-glucanase Eng16A from *coprinopsis cinerea* on barley β-glucan. Food Chem 2019; 287: 160-6. http://dx.doi.org/10.1016/j.foodchem.2019.02.086 PMID: 30857685

[78] Wang Y, Niu X, Guo X, *et al.* Heterologous expression, characterization and possible functions of the chitin deacetylases, Cda1 and Cda2, from mushroom *Coprinopsis cinerea*. Glycobiology 2018; 28(5): 318-32. http://dx.doi.org/10.1093/glycob/cwy007 PMID: 29370398

[79] Wiercigroch E, Szafraniec E, Czamara K, *et al.* Raman and infrared spectroscopy of carbohydrates: A review. Spectrochim Acta A Mol Biomol Spectrosc 2017; 185: 317-35. http://dx.doi.org/10.1016/j.saa.2017.05.045 PMID: 28599236

[80] Wang Y, Meng Z, Guo J, *et al.* Effect of wheat bran feruloyl oligosaccharides on the performance, blood metabolites, antioxidant status and rumen fermentation of lambs. Small Rumin Res 2019; 175: 65-71. http://dx.doi.org/10.1016/j.smallrumres.2019.04.006

[81] Alam MN, Bristi NJ, Rafiquzzaman M. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm J 2013; 21(2): 143-52. http://dx.doi.org/10.1016/j.jsps.2012.05.002 PMID: 24936134

[82] Hou Y, Ding X, Hou W. Composition and antioxidant activity of water-soluble oligosaccharides from *Hericium erinaceus*. Mol Med Rep 2015; 11(5): 3794-9. http://dx.doi.org/10.3892/mmr.2014.3121 PMID: 25529054

[83] Kou X, Mao C, Xie B, *et al.* Functional characterization of oligosaccharides purified from *Asparagus officinalis* peel. J Food Nutr Res 2016; 55: 313-24.

[84] Kang HJ, Jo C, Kwon JH, Son JH, An BJ, Byun MW. Antioxidant and cancer cell proliferation inhibition effect of citrus pectin-oligosaccharide prepared by irradiation. J Med Food 2006; 9(3): 313-20. http://dx.doi.org/10.1089/jmf.2006.9.313 PMID: 17004892

[85] Yuan X, Wang J, Yao H. Antioxidant activity of feruloylated oligosaccharides from wheat bran. Food Chem 2005; 90: 759-64. http://dx.doi.org/10.1016/j.foodchem.2004.05.025

[86] Sun Y, Yang B, Wu Y, *et al.* Structural characterization and antioxidant activities of κ-carrageenan oligosaccharides degraded by different methods. Food Chem 2015; 178: 311-8. http://dx.doi.org/10.1016/j.foodchem.2015.01.105 PMID: 25704717

[87] Xia Z. Preparation of the oligosaccharides derived from *Flammulina velutipes* and their antioxidant activities. Carbohydr Polym 2015; 118: 41-3. http://dx.doi.org/10.1016/j.carbpol.2014.10.074 PMID: 25542105

[88] Xiong X, Li M, Xie J, Jin Q, Xue B, Sun T. Antioxidant activity of xanthan oligosaccharides prepared by different degradation methods. Carbohydr Polym 2013; 92(2): 1166-71. http://dx.doi.org/10.1016/j.carbpol.2012.10.069 PMID: 23399142

[89] Xiong X, Li M, Xie J, Xue B, Sun T. Preparation and antioxidant activity of xanthan oligosaccharides derivatives with similar substituting degrees. Food Chem 2014; 164: 7-11. http://dx.doi.org/10.1016/j.foodchem.2014.05.001 PMID: 24996297

[90] Yao X-C, Cao Y, Wu S-J. Antioxidant activity and antibacterial activity of peach gum derived oligosaccharides. Int J Biol Macromol 2013; 62: 1-3. http://dx.doi.org/10.1016/j.ijbiomac.2013.08.022 PMID: 23973489

[91] Sun T, Zhu Y, Xie J, Yin X. Antioxidant activity of N-acyl chitosan oligosaccharide with same substituting degree. Bioorg Med Chem Lett 2011; 21(2): 798-800. http://dx.doi.org/10.1016/j.bmcl.2010.11.097 PMID: 21190855

[92] Zhao D, Wang J, Tan L, Sun C, Dong J. Synthesis of N-furoyl chitosan and chito-oligosaccharides and evaluation of their antioxidant activity *in vitro*. Int J Biol Macromol 2013; 59: 391-5. http://dx.doi.org/10.1016/j.ijbiomac.2013.04.072 PMID: 23664935

[93] Liang S, Liao W, Ma X, Li X, Wang Y. H_2O_2 oxidative preparation, characterization and antiradical activity of a novel oligosaccharide derived from flaxseed gum. Food Chem 2017; 230: 135-44. http://dx.doi.org/10.1016/j.foodchem.2017.03.029 PMID: 28407893

[94] Antov MG, Đorđević TR. Environmental-friendly technologies for the production of antioxidant xylooligosaccharides from wheat chaff. Food Chem 2017; 235: 175-80. http://dx.doi.org/10.1016/j.foodchem.2017.05.058 PMID: 28554623

[95] Gowdhaman D, Ponnusami V. Production and optimization of xylooligosaccharides from corncob by *Bacillus aerophilus* KGJ2 xylanase and its antioxidant potential. Int J Biol Macromol 2015; 79: 595-600. http://dx.doi.org/10.1016/j.ijbiomac.2015.05.046 PMID: 26038103

[96] Wu S, Huang X. Preparation and antioxidant activities of oligosaccharides from *Crassostrea gigas*. Food Chem 2017; 216: 243-6. http://dx.doi.org/10.1016/j.foodchem.2016.08.043 PMID: 27596415

[97] Jagtap S, Deshmukh RA, Menon S, Das S. Xylooligosaccharides production by crude microbial enzymes from agricultural waste without prior treatment and their potential application as nutraceuticals. Bioresour Technol 2017; 245(Pt A): 283-8. http://dx.doi.org/10.1016/j.biortech.2017.08.174 PMID: 28892703

[98] Katapodis P, Vardakou M, Kalogeris E, Kekos D, Macris BJ, Christakopoulos P. Enzymic production of a feruloylated oligosaccharide with antioxidant activity from wheat flour arabinoxylan. Eur J Nutr 2003; 42(1): 55-60. http://dx.doi.org/10.1007/s00394-003-0400-z PMID: 12594542

[99] Xu S-Y, Kan J, Hu Z, *et al.* Quantification of neoagaro-oligosaccharide production through enzymatic hydrolysis and its anti-oxidant activities. Molecules 2018; 23(6): 2-10. http://dx.doi.org/10.3390/molecules23061354 PMID: 29874799

[100] Kang OL, Ghani M, Hassan O, *et al.* Novel agaro-oligosaccharide production through enzymatic hydrolysis: physicochemical properties and antioxidant activities. Food Hydrocoll 2014; 42: 304-8. http://dx.doi.org/10.1016/j.foodhyd.2014.04.031

[101] Sen M, Atik H. The antioxidant properties of oligo sodium alginates prepared by radiation-induced degradation in aqueous and hydrogen peroxide solutions. Radiat Phys Chem 2012; 81: 816-22. http://dx.doi.org/10.1016/j.radphyschem.2012.03.025

[102] Falkeborg M, Cheong LZ, Gianfico C, *et al.* Alginate oligosaccharides: enzymatic preparation and antioxidant property evaluation. Food Chem 2014; 164: 185-94. http://dx.doi.org/10.1016/j.foodchem.2014.05.053 PMID: 24996323

[103] Jin W, Ren L, Liu B, Zhang Q, Zhong W. Structural features of sulfated glucuronomannan. Mar Drugs 2018; 16(9): 1-12. http://dx.doi.org/10.3390/md16090291 PMID: 30134603

[104] Zhao W, Chen H, Wu L, Ma W, Xie Y. Antioxidant properties of feruloylated oligosaccharides of different degrees of polymerization from wheat bran. Glycoconj J 2018; 35(6): 547-59. http://dx.doi.org/10.1007/s10719-018-9847-2 PMID: 30343348

[105] Li E, Yang S, Zou Y, *et al.* Purification, characterization, prebiotic preparations and antioxidant activity of oligosaccharides from mulberries. Molecules 2019; 24(12): 1-11. http://dx.doi.org/10.3390/molecules24122329 PMID: 31242560

[106] Shimoda K, Hamada H, Hamada H. Synthesis of xylooligosaccharides of daidzein and their anti-oxidant and anti-allergic activities. Int J Mol Sci 2011; 12(9): 5616-25. http://dx.doi.org/10.3390/ijms12095616 PMID: 22016613

[107] Apak R, Özyürek M, Güçlü K, Çapanoğlu E. Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. J Agric Food Chem 2016; 64(5): 997-1027. http://dx.doi.org/10.1021/acs.jafc.5b04739 PMID: 26728425

[108] Sing S, Singh RP. *In vitro* methods of assay of antioxidants: an overview. Food Rev Int 2008; 24: 392-415. http://dx.doi.org/10.1080/87559120802304269

[109] Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J 2012; 5(1): 9-19. http://dx.doi.org/10.1097/WOX.0b013e3182439613 PMID: 23268465

[110] Comar JF, Babeto de Sá-Nakanishi A, de Oliveira AL, *et al.* Oxidative state of the liver of rats with adjuvant-induced arthritis. Free Radic Biol Med 2013; 58: 144-53. http://dx.doi.org/10.1016/j.freeradbiomed.2012.12.003 PMID: 23246655

[111] Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem 2015; 30(1): 11-26. http://dx.doi.org/10.1007/s12291-014-0446-0 PMID: 25646037

[112] Correa VG, de Sá-Nakanishi AB, Gonçalves GA, *et al.* Yerba mate aqueous extract improves the oxidative and inflammatory states of rats with adjuvant-induced arthritis. Food Funct 2019; 10(9): 5682-96. http://dx.doi.org/10.1039/C9FO00491B PMID: 31435625

[113] Soares AA, de Sá-Nakanishi AB, Bracht A, *et al.* Hepatoprotective effects of mushrooms. Molecules 2013; 18(7): 7609-30. http://dx.doi.org/10.3390/molecules18077609 PMID: 23884116

[114] Peña-Bautista C, Vento M, Baquero M, Cháfer-Pericás C. Lipid peroxidation in neurodegeneration. Clin Chim Acta 2019; 497: 178-88. http://dx.doi.org/10.1016/j.cca.2019.07.037 PMID: 31377127

[115] Singh V, Fedeles BI, Li D, *et al.* Mechanism of repair of acrolein- and malondialdehyde-derived exocyclic guanine adducts by the α-ketoglutarate/Fe(II) dioxygenase AlkB. Chem Res Toxicol 2014; 27(9): 1619-31. http://dx.doi.org/10.1021/tx5002817 PMID: 25157679

[116] Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014; 2014: 360438. http://dx.doi.org/10.1155/2014/360438 PMID: 24999379

[117] Wang Y, Yang M, Lee S-G, *et al.* Plasma total antioxidant capacity is associated with dietary intake and plasma level of antioxidants in postmenopausal women. J Nutr Biochem 2012; 23(12): 1725-31. http://dx.doi.org/10.1016/j.jnutbio.2011.12.004 PMID: 22617460

[118] Yen CH, Kuo YW, Tseng YH, Lee MC, Chen HL. Beneficial effects of fructo-oligosaccharides supplementation on fecal bifidobacteria and index of peroxidation status in constipated nursing-home residents--a placebo-controlled, diet-controlled trial. Nutrition 2011; 27(3): 323-8. http://dx.doi.org/10.1016/j.nut.2010.02.009 PMID: 20579847

[119] Galdino FMP, Andrade MER, Barros PAV, *et al.* Pretreatment and treatment with fructo-oligosaccharides attenuate intestinal mucositis induced by 5-FU in mice. J Funct Foods 2018; 49: 485-92. http://dx.doi.org/10.1016/j.jff.2018.09.012

[120] Faseleh Jahromi M, Shokryazdan P, Idrus Z, Ebrahimi R, Liang JB. *In Ovo* and dietary administration of oligosaccharides extracted from palm kernel cake influence general health of pre- and neonatal broiler chicks. PLoS One 2017; 12(9): e0184553. http://dx.doi.org/10.1371/journal.pone.0184553 PMID: 28880894

[121] Qu Y, Wang Z, Zhou H, Kang M, Dong R, Zhao J. Oligosaccharide nanomedicine of alginate sodium improves therapeutic results of posterior lumbar interbody fusion with cages for degenerative lumbar disease in osteoporosis patients by downregulating serum miR-155. Int J Nanomedicine 2017; 12: 8459-69. http://dx.doi.org/10.2147/IJN.S143824 PMID: 29200854

[122] Chen J, Hu Y, Zhang L, *et al.* Alginate oligosaccharide DP5 exhibits antitumor effects in osteosarcoma patients following surgery. Front Pharmacol 2017; 8: 623. http://dx.doi.org/10.3389/fphar.2017.00623 PMID: 28955228

[123] Wan J, Zhang J, Chen D, *et al.* Effects of alginate oligosaccharide on the growth performance, antioxidant capacity and intestinal digestion-absorption function in weaned pigs. Anim Feed Sci Technol 2017; 234: 118-27. http://dx.doi.org/10.1016/j.anifeedsci.2017.09.006

[124] Lu J, Qi C, Mchele S, *et al.* Dietary mannan oligosaccharide (MOS) improves growth performance, antioxidant capacity, non-specific immunity and intestinal histology of juvenile Chinese mitten crabs (*Eriocheir sinensis*). Aquaculture 2019; 510: 337-46. http://dx.doi.org/10.1016/j.aquaculture.2019.05.048

[125] Cheng Y, Du M, Xu Q, Chen Y, Wen C, Zhou Y. Dietary mannan oligosaccharide improves growth performance, muscle oxidative status, and meat quality in broilers under cyclic heat stress. J Therm Biol 2018; 75: 106-11. http://dx.doi.org/10.1016/j.jtherbio.2018.06.002 PMID: 30017045

[126] Wang Z, Yu H, Xie J, Cui H, Gao X. Effect of pectin oligosaccharides and zinc chelate on growth performance, zinc status, antioxidant ability, intestinal morphology and short-chain fatty acids in broilers. J Anim Physiol Anim Nutr (Berl) 2019; 103(3): 935-46. http://dx.doi.org/10.1111/jpn.13076 PMID: 30801843

[127] Xie C, Wu X, Long C, *et al.* Chitosan oligosaccharide affects antioxidant defense capacity and placental amino acids transport of sows. BMC Vet Res 2016; 12(1): 243-50. http://dx.doi.org/10.1186/s12917-016-0872-8 PMID: 27806719

[128] Jiang T, Xing X, Zhang L, *et al.* Clinical study chitosan oligosaccharides show protective effects in coronary heart disease by improving antioxidant capacity *via* the increase in intestinal probiotics. 2019.

[129] Guan D, Sun H, Meng X, *et al.* Effects of different molar mass chitooligosaccharides on growth, antioxidant capacity, non-specific immune response, and resistance to *Aeromonas hydrophila* in GIFT tilapia *Oreochromis niloticus*. Fish Shellfish Immunol 2019; 93: 500-7. http://dx.doi.org/10.1016/j.fsi.2019.08.001 PMID: 31377430

[130] Kong SZ, Li JC, Li SD, *et al.* Anti-aging effect of chitosan oligosaccharide on D-galactose-induced subacute aging in mice. Mar Drugs 2018; 16(6): 1-13. http://dx.doi.org/10.3390/md16060181 PMID: 29794973

[131] Fang IM, Yang CM, Yang CH. Chitosan oligosaccharides prevented retinal ischemia and reperfusion injury *via* reduced oxidative stress and inflammation in rats. Exp Eye Res 2015; 130: 38-50. http://dx.doi.org/10.1016/j.exer.2014.12.001 PMID: 25479043

[132] Qu D, Han J. Investigation of the antioxidant activity of chitooligosaccharides on mice with high-fat diet. Rev Bras Zootec 2016; 11: 661-6. http://dx.doi.org/10.1590/s1806-92902016001100004

[133] Zhang H, Wang J, Liu Y, Sun B. Wheat bran feruloyl oligosaccharides modulate the phase II detoxifying/antioxidant enzymes *via* Nrf2 signaling. Int J Biol Macromol 2015; 74: 150-4. http://dx.doi.org/10.1016/j.ijbiomac.2014.12.011 PMID: 25542177

[134] Wang J, Sun B, Cao Y, *et al.* Wheat bran feruloyl oligosaccharides enhance the antioxidant activity of rat plasma. Food Chem 2010; 123: 472-6. http://dx.doi.org/10.1016/j.foodchem.2010.05.033

[135] Zhang H, Zhang S, Wang J, *et al.* Wheat bran feruloyl oligosaccharides protect against AAPH-induced oxidative injury *via* p38MAPK/PI3K-Nrf2/Keap1-MafK pathway. J Funct Foods 2017; 29: 53-9. http://dx.doi.org/10.1016/j.jff.2016.12.009

[136] Krishna G, Muralidhara. Oral supplements of combined fructo- and xylo-oligosaccharides during perinatal period significantly offsets acrylamide-induced oxidative impairments and neurotoxicity in rats. J Physiol Pharmacol 2018; 69(5): 801-14. PMID: 30683831

[137] Liu XY, Liu D, Lin GP, *et al.* Anti-ageing and antioxidant effects of sulfate oligosaccharides from green algae *Ulva lactuca* and *Enteromorpha prolifera* in SAMP8 mice. Int J Biol Macromol 2019; 139: 342-51. http://dx.doi.org/10.1016/j.ijbiomac.2019.07.195 PMID: 31377292

[138] Wang H, Liu YM, Qi ZM, *et al.* An overview on natural polysaccharides with antioxidant properties. Cur Med Chem 2013; 2899-913.

[139] Wang J, Hu S, Nie S, *et al.* Reviews on mechanisms of *in vitro* antioxidant activity of polysaccharides. In: Oxi Med Cell Longev. 2016; 13: 5692852.

[140] Priyan Shanura Fernando I, Kim K-N, Kim D, Jeon YJ. Algal polysaccharides: potential bioactive substances for cosmeceutical applications. Crit Rev Biotechnol 2018; 9: 1-15. PMID: 30198346

[141] Jeong HK, Lee D, Kim HP, Baek SH. Structure analysis and antioxidant activities of an amylopectin-type polysaccharide isolated from dried fruits of Terminalia chebula. Carbohydr Polym 2019; 211: 100-8. http://dx.doi.org/10.1016/j.carbpol.2019.01.097 PMID: 30824068

[142] Li S, Xiong Q, Lai X, *et al.* Molecular modification of polysaccharides and resulting bioactivities. Compr Rev Food Sci 2016; 15: 237-50. http://dx.doi.org/10.1111/1541-4337.12161

[143] Liu J, Wilfor S, Xu CA. Review of bioactive plant polysaccharides: biological activities, functionalization, and biomedical applications. Bioact Carbohydr Dietary Fibre 2015; 5: 31-61. http://dx.doi.org/10.1016/j.bcdf.2014.12.001

[144] Khan BM, Qiu HM, Xu SY, Liu Y, Cheong KL. Physicochemical characterization and antioxidant activity of sulphated polysaccharides derived from *Porphyra haitanensis*. Int J Biol Macromol 2019; 145: 1155-61. http://dx.doi.org/10.1016/j.ijbiomac.2019.10.040 PMID: 31730957

[145] Chen HM, Yan XJ. Antioxidant activities of agaro-oligosaccharides with different degrees of polymerization in cell-based system. Biochim Biophys Acta 2005; 1722(1): 103-11. http://dx.doi.org/10.1016/j.bbagen.2004.11.016 PMID: 15716131

[146] Chen HM, Zheng L, Yan XJ. The preparation and bioactivity research of agaro-oligosaccharides. Food Technol Biotechnol 2005; 43: 29-36.

[147] Je JY, Park PJ, Kim SK. Free radical scavenging properties of hetero-chitooligosaccharides using an ESR spectroscopy. Food Chem Toxicol 2004; 42(3): 381-7. http://dx.doi.org/10.1016/j.fct.2003.10.001 PMID: 14871580

[148] Kang L, Zhang X, Wang R, *et al.* β-Glucosidase BGL1 from *Coprinopsis cinerea* exhibits a distinctive hydrolysis and transglycosylation activity for application in the production of 3-O-β-D-gentiobiosyl-D-laminarioligosaccharides. J Agric Food Chem 2019; 67(38): 10744-55. http://dx.doi.org/10.1021/acs.jafc.9b04488 PMID: 31525900

[149] Liu Z, Xiong Y, Yi L, *et al.* Endo-β-1,3-glucanase digestion combined with the HPAEC-PAD-MS/MS analysis reveals the structural differences between two laminarins with different bioactivities. Carbohydr Polym 2018; 194: 339-49. http://dx.doi.org/10.1016/j.carbpol.2018.04.044 PMID: 29801847

[150] Eom TK, Senevirathne M, Kim SK. Synthesis of phenolic acid conjugated chitooligosaccharides and evaluation of their antioxidant activity. Environ Toxicol Pharmacol 2012; 34(2): 519-27. http://dx.doi.org/10.1016/j.etap.2012.05.004 PMID: 22809749

[151] Ngo D-H, Qian Z-J, Vo T-S, *et al.* Antioxidant activity of gallate-chitooligosaccharides in mouse macrophage RAW264-7 cells. Carbohydr Polym 2011; 84: 1282-8. http://dx.doi.org/10.1016/j.carbpol.2011.01.022

[152] Yuan H, Zhang W, Li X, *et al.* Preparation and *in vitro* antioxidant activity of κ-carrageenan oligosaccharides and their oversulfated, acetylated, and phosphorylated derivatives. Carbohydr Res 2005; 340(4): 685-92. http://dx.doi.org/10.1016/j.carres.2004.12.026 PMID: 15721341

[153] Bian J, Peng F, Peng XP, Peng P, Xu F, Sun RC. Structural features and antioxidant activity of xylooligosaccharides enzymatically produced from sugarcane bagasse. Bioresour Technol 2013; 127: 236-41. http://dx.doi.org/10.1016/j.biortech.2012.09.112 PMID: 23131647

[154] Khanafari A, Marandi R, Sanatei S. Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods. Iran J Environ Health Sci Eng 2008; 5: 19-24.

[155] Lodhi G, Kim Y-S, Hwang J-W, *et al.* Chitooligosaccharide and its derivatives: preparation and biological applications. In: BioMed Res Int 2014. 654913.

[156] Phil L, Naveed M, Mohammad IS, Bo L, Bin D. Chitooligosaccharide: An evaluation of physicochemical and biological properties with the proposition for determination of thermal degradation products. Biomed Pharmacother 2018; 102: 438-51. http://dx.doi.org/10.1016/j.biopha.2018.03.108 PMID: 29579704

[157] Yuan W-P, Liu B, Liu C-H, *et al.* Antioxidant activity of chito-oligosaccharides on pancreatic islet cells in streptozotocin-induced diabetes in rats. World J Gastroenterol 2009; 15(11): 1339-45. http://dx.doi.org/10.3748/wjg.15.1339 PMID: 19294763

[158] Fernandes JC, Eaton P, Nascimento H, *et al.* Antioxidant activity of chitooligosaccharides upon two biological systems: Erythrocytes and bacteriophages. Carbohydr Polym 2010; 79: 1101-6. http://dx.doi.org/10.1016/j.carbpol.2009.10.050

[159] Ngo D-H, Kim S-K. Antioxidant effects of chitin, chitosan, and their derivatives. Adv Food Nutr Res 2014; 73: 15-31. http://dx.doi.org/10.1016/B978-0-12-800268-1.00002-0 PMID: 25300540

[160] Sen VD, Sokolova EM, Neshev NI, *et al.* Low molecular chitosan (poly) nitroxides: synthesis and evaluation as antioxidants on free radical-induced erythrocyte hemolysis. React Funct Polym 2017; 111: 53-9. http://dx.doi.org/10.1016/j.reactfunctpolym.2016.12.006

[161] Zhou J, Dai R, Wang Y, *et al.* A novel thermophilic exochitinase ChiEn3 from *Coprinopsis cinerea* exhibits a hyperhydrolytic activity toward 85% deacetylated chitosan and a significant application to preparation of chitooligosaccharides from the chitosan. Carbohydr Polym 2019; 207: 729-36. http://dx.doi.org/10.1016/j.carbpol.2018.12.047 PMID: 30600059

[162] Veenashri BR, Muralikrishna G. *In vitro* antioxidant activity of xylooligosaccharides derived from cereal and millet brans: a comparative study. Food Chem 2011; 126: 1475-81. http://dx.doi.org/10.1016/j.foodchem.2010.11.163

[163] Wang J, Sun B, Cao Y, Tian Y. Protection of wheat bran feruloyl oligosaccharides against free radical-induced oxidative damage in normal human erythrocytes. Food Chem Toxicol 2009; 47(7): 1591-9. http://dx.doi.org/10.1016/j.fct.2009.04.006 PMID: 19371769

[164] Nizami AS, Rehan M, Waqas M, *et al.* Waste biorefineries: Enabling circular economies in developing countries. Bioresour Technol 2017; 241: 1101-17. http://dx.doi.org/10.1016/j.biortech.2017.05.097 PMID: 28579178

[165] Ngo DH, Wijesekara I, Vo TS, *et al.* Marine food-derived functional ingredients as potential antioxidants in the food industry: An overview. Food Res Int 2011; 44: 523-9. http://dx.doi.org/10.1016/j.foodres.2010.12.030

[166] Wang P, Jiang X, Jiang Y, *et al. In vitro* antioxidative activities of three marine oligosaccharides. Nat Prod Res 2007; 21(7): 646-54. http://dx.doi.org/10.1080/14786410701371215 PMID: 17613823

[167] Chen H, Yan X, Zhu P, Lin J. Antioxidant activity and hepatoprotective potential of agaro-oligosaccharides *in vitro* and *in vivo*. Nutr J 2006; 5: 31-42. http://dx.doi.org/10.1186/1475-2891-5-31 PMID: 17140450

[168] Rivas S, Conde E, Moure A, Domínguez H, Parajó JC. Characterization, refining and antioxidant activity of saccharides derived

from hemicelluloses of wood and rice husks. Food Chem 2013; 141(1): 495-502. http://dx.doi.org/10.1016/j.foodchem.2013.03.008 PMID: 23768385

[169] Samanta AK, Jayapal N, Kolte AP, *et al.* Enzymatic production of xylooligosaccharides from alkali solubilized xylan of natural grass (*Sehima nervosum*). Bioresour Technol 2012; 112: 199-205. http://dx.doi.org/10.1016/j.biortech.2012.02.036 PMID: 22414575

[170] Wu SC, Wen TN, Pan CL. Algal-oligosaccharide-lysates prepared by two bacterial agarases stepwise hydrolyzed and their antioxidative properties. Fish Sci 2005; 71: 1149-59. http://dx.doi.org/10.1111/j.1444-2906.2005.01075.x

[171] Zhang Y, Wang S, Xu W, *et al.* Valorization of lignin-carbohydrate complexes from hydrolysates of norway spruce: efficient separation, structural characterization, and antioxidant activity. ACS Sustain Chem& Eng 2018; 7: 1447-56. http://dx.doi.org/10.1021/acssuschemeng.8b05142

[172] Linchongkon K, Khumijitjaru P, Wiboonsirikul J, *et al.* Extraction of oligosaccharides from passion fruit peel by subcritical water treatment. J Food Process Eng 2017; 40e12269 http://dx.doi.org/10.1111/jfpe.12269

[173] Rodríguez-Seoane P, Díaz-Reinoso B, González-Muñoz MJ, *et al.* Innovative technologies for the extraction of saccharides and phenolic fractions from *Pleurotus eryngii*. Lebensm Wiss Technol 2019; 2019(101): 774-82. http://dx.doi.org/10.1016/j.lwt.2018.11.062

[174] Bubalo MC, Vidović S, Redovniković IR, *et al.* New perspective in extraction of plant biologically active compounds by green solvents. Food Bioprod Process 2018; 109: 52-73. http://dx.doi.org/10.1016/j.fbp.2018.03.001

[175] Mena-Garcia A, Ruiz-Matute AI, Soria AC, *et al.* Green techniques for extraction of bioactive carbohydrates. Trends Analyt Chem 2019; 119115612 http://dx.doi.org/10.1016/j.trac.2019.07.023