UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS
Programa de Pós-Graduação em Ciência de Alimentos

Produção e caracterização de xilooligossacarídeos antioxidantes obtidos de resíduos de pupunheira

TATIANE FRANCIELLI VIEIRA

Maringá
2021

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"PRODUÇÃO E CARACTERIZAÇÃO DE XILOOLIGOSSACARÍDEOS ANTIOXIDANTES OBTIDOS DE RESÍDUOS DE PUPUNHEIRA"

Tese apresentada à Universidade Estadual de Maringá, como parte das exigências do Programa de Pós-graduação em Ciência de Alimentos para a obtenção do grau de Doutora em Ciência de Alimentos.

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Maringá
2021

Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá - PR, Brasil)

V658p

Vieira, Tatiane Francielli

Produção e caracterização de xilooligossacarídeos antioxidantes obtidos de resíduos de pupunheira / Tatiane Francielli Vieira. -- Maringá, PR, 2021.
90 f.color., figs., tabs.

Orientadora: Profa. Dra. Rosane Marina Peralta.
Tese (Doutorado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Programa de Pós-Graduação em Ciência de Alimentos, 2021.

1. Antioxidante. 2. *Bactris gasipaes Kunth*. 3. Oligossacarídeo. 4. Economia circular. 5. Recuperação de resíduo. I. Peralta, Rosane Marina, orient. II. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Programa de Pós-Graduação em Ciência de Alimentos. III. Título.

CDD 23.ed. 664

Jane Lessa Monção - CRB 9/1173

Orientadora

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BIOGRAFIA

Tatiane Francielli Vieira nasceu em 26/03/1993 na cidade de Curitiba-PR.

Possui graduação em Engenharia de Alimentos e mestrado em Tecnologia de Alimentos, ambos pela Universidade Tecnológica Federal do Paraná (UTFPR).

Tem experiência na área de Ciência e Tecnologia de Alimentos, atuando principalmente no aproveitamento de resíduos da agroindústria, na caracterização e na determinação de atividade antioxidante de compostos bioativos.

Ingressou no Programa de Pós-graduação em Ciência de Alimentos da Universidade Estadual de Maringá para obtenção do título de doutora em março de 2018.

Além dos trabalhos que compõem sua tese de doutorado, participou de trabalhos de pesquisa desenvolvidos no Laboratório de Bioquímica de Microrganismos e de Alimentos, tendo co-autoria em 3 artigos científicos e 3 capítulos de livro publicados no período de 2018-2020.

Dedico

A minha família, ao meu noivo e aos amigos pelo apoio.

AGRADECIMENTOS

Agradeço primeiramente a Deus, pela vida, por me permitir manter a fé e a esperança, me guiando em mais uma jornada.

A minha orientadora professora Dra. Rosane Marina Peralta, pela oportunidade de realizar esse trabalho, pela valiosa orientação, ensinamentos, paciência e confiança em mim depositada durante esse período.

Aos meus pais, Sônia Maria Vieira e Natal Vieira e à minha tia Sandra Garcia Neves, em especial, e aos demais familiares e amigos, por todo incentivo, carinho e pelas palavras por vezes confortantes que me fizeram ter esperança, por me motivarem e me orientarem sempre a seguir o melhor caminho, comemorando sempre minhas conquistas.

Ao meu noivo Mário Landi Neto, por sempre acreditar em mim, estando ao meu lado em todos os momentos, pela paciência, amor, carinho, companheirismo e atenção que dedicou a mim.

Aos professores Dra. Rúbia Carvalho Gomes Corrêa (UniCesumar), Dra. Rosely Aparecida Peralta (UFSC), Dra. Regina de Fátima Peralta-Muniz-Moreira (UFSC), Dr. Adelar Bracht (UEM) e aos pesquisadores da Embrapa Florestas, Dr. Edson Alves de Lima e Dra. Cristiane Vieira Helm, pela parceria na realização dos experimentos e na redação dos artigos.

Às colegas de laboratório com quem convivi durante este período, pelo companheirismo, ajuda e momentos de descontração, em especial a Vanesa Gesser Correa e a Jéssica A. A. Garcia, que me acolheram e me auxiliaram no laboratório sempre que preciso.

À todos os professores e servidores do Programa de Pós-Graduação em Ciência de Alimentos da UEM.

À Coordenação de Aperfeiçoamento de Pessoal em Nível Superior (CAPES) e à Fundação Araucária, pela concessão da bolsa de pesquisa.

À todos que indiretamente contribuíram para a realização e enriquecimento dessa pesquisa.

APRESENTAÇÃO

Essa tese de doutorado é composta por dois trabalhos, um artigo de revisão e um artigo experimental:

Vieira, T. F.; Corrêa, R. C. G.; Peralta, R. A.; Moreira, R. de F. P. M.; Bracht, A.; Peralta, R. M. An Overview of Structural Aspects and Health Beneficial Effects of Antioxidant Oligosaccharides. **Current Pharmaceutical Design**, v. 26, n. 16, p. 1759-1777, 2020.

Vieira, T. F.; Corrêa, R. C. G.; Moreira, R. de F. P. M.; Peralta, R. A.; Lima, E. A. de; Helm, C. V.; Garcia, J. A. A.; Bracht, A.; Peralta, R. M. Valorization of peach palm (*Bactris gasipaes* Kunth) waste: production of antioxidant xylooligosaccharides. **Waste and Biomass Valorization**, submetido.

GENERAL ABSTRACT

INTRODUCTION AND AIMS: Antioxidants are natural or synthetic substances that can prevent or delay, on a substrate, oxidative cell damage caused by physiological oxidants. Research carried out recently in the field of natural antioxidants has considerably expanded the knowledge on naturally occurring compounds with beneficial health effects in foods, among which oligosaccharides can be highlighted. These are of considerable interest due to their physicochemical and biological properties, especially as antioxidants, antimicrobials and prebiotics. Oligosaccharides can be found naturally in some foods or produced from the depolymerization of polysaccharides, such as xylans, from which xylooligosaccharides (XOS) are produced via hydrolysis. Xylans can be extracted from lignocellulosic materials through several kinds of pre-treatments. In Brazil, the production and consumption of palm heart (palmito), especially from the *Bactris gasipaes* Kunth species, generate a large amount of lignocellulosic by-products. It is known that approximately 37% of the volume of all the plant matter that arrives at the industry ends as waste generated during the processing of palm hearts. Taking into account these informations, this work has two main purposes. The first one is based on published research and aims at providing an up-to-date, broad and critical review on the topic of antioxidant oligosaccharides. The second purpose is of experimental nature and consists in a study aiming at adding value to the peach palm residues through the production of xylooligosaccharides. Besides determining its structural features, the production of the xylooligosaccharides will be accompanied by an investigation of its antioxidant properties, which can be regarded as indicators of its possible health beneficial properties.

MATERIAL AND METHODS: For the review article, a search, using the words oligosaccharides and antioxidants, was conducted for recent studies trying to establish, whenever possible, correlations between chemical structure and antioxidant activity. Consideration was given to both in vitro and in vivo methods in animals and humans. For the experimental study, peach palm wastes (inner sheath and peel) were used, kindly donated by Embrapa Florestas, Colombo - PR. An alkaline extraction (NaOH, 8%) was performed to obtain xylans, which were characterized by techniques of Fourier transform infrared spectroscopy, thermogravimetric analysis and scanning electron microscopy.

From the extracted xylans, xylooligosaccharides were produced via enzymatic hydrolysis, using a commercially available endoxylanase (1.25 $U.mL^{-1}$) from *Aspergillus oryzae.* In parallel, XOS were produced from commercial xylans (beechwood, birchwood, oat spelt), for comparative purposes. The XOS yield was measured using the gravimetric method and evaluation of reducing sugar contents by the DNS method, in addition to their detection by thin layer chromatography. The antioxidant potential of the XOS were evaluated employing in vitro assays: TPC, FRAP, ORAC, scavenging of hydroxyl radicals and DPPH. Finally, the XOS were characterized by mass spectrometry (ESI-MS). All data were submitted to ANOVA with a Student-Newman-Keuls post hoc test, using the GraphPad Prism software (version 8.0).

RESULTS AND DISCUSSION: According to the survey conducted, it was noted that the oligosaccharides most studied to date include chitooligosaccharides, xylooligosaccharides, and their derivatives. The avaliable data reveal that the antioxidant activities of oligosaccharides depend on several factors, such as degree of polymerization, the method used for depolymerization, solubility, monosaccharide composition, the type of glycosidic bonds of the side chains, molecular weight, the content of reducing sugar, and the presence of phenolic groups such as ferulic acid and uronic acid, among others. Besides, the addition of organic groups to the structures of oligosaccharides allows to modify the antioxidant capacity of these compounds, increasing the spectrum of potentially useful molecules. For the experimental study, xylans from peach palm inner sheath and peel were extracted using an alkaline treatment and yields of 82% and 80% were achieved, respectively. The XOS obtained through enzymatic hydrolysis showed yields of 50.1% and 48.8%, for the inner sheath and peel xylans, respectively. The antioxidant potentials of XOS were evaluated by five in vitro assays and, in overall terms, the XOS of the xylans from the peach palm wastes, peel especially, showed higher antioxidant potential when compared to the XOS obtained from commercial xylans. The ESI-MS spectra suggest XOS with grouped xylose or arabinose units ranging from 2 to 5 (differing by 132 Da) and as sodium adduct ions $[M + Na]^+$ in the range of 100-1000 m/z.

CONCLUSIONS: From the literature review it can be concluded that the large-scale production of antioxidant oligosaccharides is still a challenge for food science and technology. However, several methods have recently been developed, modified, and adapted to optimize the production of different oligosaccharides. Besides, a great amount

of high-quality evidence has been accumulated during the last decade in support of meaningful antioxidant activity of oligosaccharides and derivatives. This suggests that the ingestion of antioxidant oligosaccharides can be viewed as beneficial to human and animal health. Regarding the capacity of free radical scavenging, in general, the XOS obtained from the xylans of the peach palm wastes showed higher antioxidant capacity than the XOS obtained from the three commercial xylans. These results suggest that the peach palm wastes can be explored for the production of XOS, which could be applied as natural antioxidants in functional foods and pharmaceutical preparations. It is worth to emphasize that this application, by being linked to the bio-based economy, will certainly fit well into the biocircular economy concept.

Key words: antioxidant, oligosaccharides, *Bactris gasipaes* Kunth, waste recovery, circular economy.

RESUMO GERAL

INTRODUÇÃO E OBJETIVOS: Antioxidantes são substâncias naturais ou sintéticas que podem prevenir ou retardar, em um substrato, o dano celular oxidativo causado por oxidantes fisiológicos. Pesquisas realizadas recentemente na área de antioxidantes naturais expandiram consideravelmente o conhecimento sobre compostos de ocorrência natural com efeitos benéficos para a saúde em alimentos, podendo-se destacar dentre estes, os oligossacarídeos. Estes apresentam considerável interesse devido às suas propriedades físico-químicas e biológicas, especialmente como antioxidantes, antimicrobianas e prebióticas. Os oligossacarídeos podem ser encontrados naturalmente em alguns alimentos ou produzidos a partir da despolimerização de polissacarídeos, tal como xilanos, a partir dos quais os xilooligossacarídeos (XOS) são produzidos via hidrólise. Xilanos podem ser extraídos de materiais lignocelulósicos por diversos tipos de pré-tratamentos. No Brasil, a produção e o consumo do palmito, principalmente da espécie *Bactris gasipaes* Kunth, gera grande quantidade de resíduos lignocelulósicos. Sabe-se que aproximadamente 37% do volume de toda a matéria vegetal que chega à indústria acaba como resíduo gerado durante o processamento do palmito. Levando em consideração essas informações, este trabalho tem dois objetivos principais. O primeiro é baseado em pesquisas publicadas e visa fornecer uma revisão atualizada, ampla e crítica sobre o tema dos oligossacarídeos antioxidantes. O segundo objetivo é de natureza experimental e consiste em um estudo visando agregar valor aos resíduos da pupunheira por meio da produção de xilo-oligossacarídeos. Além de determinar suas características estruturais, a produção dos xilo-oligossacarídeos será acompanhada por uma investigação de suas propriedades antioxidantes, que podem ser consideradas indicadoras de suas possíveis propriedades benéficas à saúde.

MATERIAL E MÉTODOS: Para o artigo de revisão, foi realizada uma busca, utilizando as palavras oligossacarídeos e antioxidantes, de estudos recentes buscando estabelecer, sempre que possível, correlações entre a estrutura química e a atividade antioxidante. Foram considerados os métodos *in vitro* e *in vivo* em animais e humanos. Para o estudo experimental, utilizaram-se os resíduos da pupunheira (bainha interna e casca), gentilmente doados pela Embrapa Florestas, Colombo - PR. Uma extração alcalina (NaOH, 8 %) foi realizada para a obtenção dos xilanos dos resíduos, os quais

foram caracterizados pelas técnicas de espectroscopia de infravermelho com transformada de Fourier, análise termogravimétrica e microscopia eletrônica de varredura. A partir dos xilanos extraídos, produziram-se os xilo-oligossacarídeos via hidrólise enzimática, empregando-se uma endoxilanase (1,25 $U.mL^{-1}$) comercialmente disponível de *A. oryzae*. Em paralelo, foram produzidos XOS a partir de xilanos comerciais (beechwood, birchwood, oat spelt), para fins comparativos. O rendimento de XOS foi medido através do método gravimétrico e da avaliação do teor de açúcares redutores pelo método DNS, além da detecção destes pela técnica de cromatografia em camada delgada. O potencial antioxidante dos XOS foi avaliado empregando-se ensaios *in vitro*: TPC, FRAP, ORAC, atividade de eliminação dos radicais hidroxila e DPPH. Por fim, os XOS foram caracterizados pela técnica de espectrometria de massas (ESI-MS). Todos os dados foram submetidos à ANOVA com teste post hoc de Student-Newman-Keuls, no software GraphPad Prism (versão 8.0).

RESULTADOS E DISCUSSÃO: A partir do levantamento realizado, notou-se que os oligossacarídeos mais estudados até o momento compreendem os quito-oligossacarídeos e os xilo-oligossacarídeos e seus derivados. Os dados disponíveis revelam que as atividades antioxidantes dos oligossacarídeos dependem de diversos fatores, como grau de polimerização, método utilizado para a despolimerização, a solubilidade, a composição do monossacarídeo, o tipo de ligações glicosídicas das cadeias laterais, o peso molecular, o teor de açúcar redutor e a presença de grupos fenólicos como ácido ferúlico e ácido urônico, entre outros. Além disso, a adição de grupos orgânicos às estruturas dos oligossacarídeos permite modificar a capacidade antioxidante destes compostos, aumentando ainda o espectro de moléculas potencialmente úteis. No estudo experimental, xilanos da bainha interna e da casca da pupunheira foram extraídos usando um tratamento alcalino e rendimentos de 82% e 80% foram alcançados, respectivamente. Os XOS obtidos via hidrólise enzimática apresentaram rendimentos de 50,1 e 48,8%, para os xilanos da bainha interna e da casca, respectivamente. Os potenciais antioxidantes dos XOS foram avaliados por cinco ensaios *in vitro* e, de modo geral, pode-se dizer que os XOS dos xilanos dos resíduos da pupunheira, em especial da casca, apresentaram maior potencial antioxidante quando comparado ao dos XOS obtidos dos xilanos comerciais. Os espectros de ESI-MS sugerem XOS com unidades agrupadas de xilose ou arabinose variando de 2 a 5 (diferindo por 132 Da) e como íons adutos de sódio $[M + Na]^+$ na faixa de 100-1000 m/z.

CONCLUSÕES: A partir da revisão de literatura, pode-se concluir que a produção em larga escala de oligossacarídeos antioxidantes ainda é um desafio para a ciência e tecnologia de alimentos. Contudo, vários métodos foram recentemente desenvolvidos, modificados e adaptados para otimizar a produção de diferentes oligossacarídeos. Além disso, uma grande quantidade de evidências de alta qualidade tem sido acumulada durante a última década em apoio a uma atividade antioxidante significativa de oligossacarídeos e derivados, sugerindo que a ingestão de oligossacarídeos antioxidantes pode ser visualizada como benéfica para a saúde humana e animal. Com relação a capacidade de eliminação de radicais livres, de modo geral, os XOS obtidos dos xilanos dos resíduos da pupunheira apresentaram maior capacidade antioxidante do que os XOS obtidos dos três xilanos comerciais. Esses resultados sugerem que os resíduos da pupunheira podem ser explorados para a produção de XOS, podendo ser aplicados como antioxidantes naturais em alimentos funcionais e preparações farmacêuticas. Vale ressaltar que esta aplicação, por estar vinculada à economia de base biológica, certamente se enquadrará bem no conceito de economia biocircular.

Palavras chaves: antioxidante, oligossacarídeos, *Bactris gasipaes* Kunth, aproveitamento de resíduos, economia circular.

An overview of structural aspects and health beneficial effects of antioxidant oligosaccharides

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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 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 on 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 to a meaningful antioxidant activity of oligosaccharides and derivatives. Ingestion of antioxidant oligosaccharides can be visualized as beneficial to human and animal health.

Key words: antioxidant, circular economy, functional properties, oligosaccharides, oligosaccharide derivatives, oxidative stress.

1. ANTIOXIDANTS: GENERAL ASPECTS AND CLASSIFICATION

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 proves 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 defence 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 compounds plays an essential preventive role towards 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 defences. The triad of first line defence enzymes, namely superoxide

dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), plays a vital role in the entire antioxidant defence strategy, particularly in what regards the super oxide 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 defence, consisting of glutathione reductase and glucose-6-phosphate dehydrogenase, does not neutralize free radicals directly; however, this system has supporting roles to 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.

Insert Figure 1

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 total of papers holding the terms ‘polysaccharide’ versus ‘antioxidant’ (5060 publications) and 'polyphenol' versus 'antioxidant' (12423 publications) corresponded to more than 9-fold and 23-fold, 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 auto-hydrolysis, 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 a 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, what 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].

Insert Figure 2

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 regio-selectivity 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

To establish 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 detects 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 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 make data interpretation difficult [71].

Recent studies have employed mass spectrometry associated to 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 them (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 evaluation of their antioxidant properties by different *in vitro* methods.

Insert Table 1

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, what 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.

Insert Table 2

3.3. Structural features of oligosaccharides determining antioxidant activity

Contaminants, especially phenolics and proteins, have been many times considered the reason of 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 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 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 has 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 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 centred 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 for 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 chito-oligosaccharide (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 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.

Insert Figure 3

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.

Insert Figure 4

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 food industry [81,100].

4. PERSPECTIVES

In recent years, the concept of linear economy has been replaced by the concept of circular economy, since the linear model is based primarily on the use of non-renewable fossil resources. The concept of 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 frequentlly in the obtainement 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, what can lead to degradation of thermolabile oligosaccharides. Additionally, other advanced techniques can be routinelly 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 [175,176] and will certainly be useful in the obtainment of antioxidant oligosaccharides.

5. 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 about the antioxidant properties of non-digestible oligosaccharides. The production in large scale of antioxidant oligosaccharides is still a challenge to food science and technology. However, several methods have been recently developed, modified, and adapted to optimise the production of different oligosaccharides. The food

industry needs more efficient, simple, and less expensive processes for their application on large scale.

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

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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.

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

Source and obtainment	Most important observations and conclusions	Ref.
Extraction		
Hot water extraction of oligosaccharide 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 oligosaccharides 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 oligosaccharides 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	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-	[89]

prepared by reacting xanthan oligosaccharides with maleic anhydride and phthalic anhydride, respectively.	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.	
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]
N-Maleoyl chitosan oligosaccharide (NMCOS) and N-succinyl chitosan oligosaccharide (NSCOS) were prepared from a chitosan oligo-saccharide by acylation with maleic anhydride and succinic anhydride, respectively.	The antioxidant activities of the derivatives were evaluated by superoxide anion $O_2^{\cdot -}$ 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 (NF-COS) 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▪ 82.58%, DPPH▪ 52.74% and ABTS▪ 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 amansii* 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 obtained 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]
Alginate oligosaccharides (AOS) were prepared from alginate using alginate lyase. The AOs were structurally characterized by thin layer chromatography, infrared spectrometry, and mass spectrometry. 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 ABTṠ, 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 polysaccharides 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 glucuronomannan 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 polysaccharide 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 oligosaccharides (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-glucopyranoside 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]

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

Oligosaccharides and experimental systems	**Most important observations and conclusions**	**Ref.**
Fructo-oligosaccharides (FOS)		
The effects of fructo-oligosaccharides (FOS) on fecal bifidobacteria, 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 polymerization were produced and purified by size-exclusion chromatography. Only one AOS (DP5) had antitumor effects on osteosarcoma cells. Osteosarcoma 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.	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	[123]

Growth performance, antioxidant capacity and intestinal digestion-absorption function in weaned pigs were evaluated and compared with a control group.	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.	
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]
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	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	129]

resistance to *Aeromonas hydrophila* in GIFT tilapia (*Oreochromis niloticus*) were evaluated.	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.	
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 cardiotoxicity, 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-activated protein kinases) phosphorylation in a rat model of retinal I/R injury induced by transiently raising the intraocular 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]
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 enzymes: 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 performance, 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) protect against 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH)	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	[135]

induced oxidant injury were investigated in rats.	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.	
The objective of this study was to evaluate the protective effect of a combination 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 *Desulfovibrio* 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]

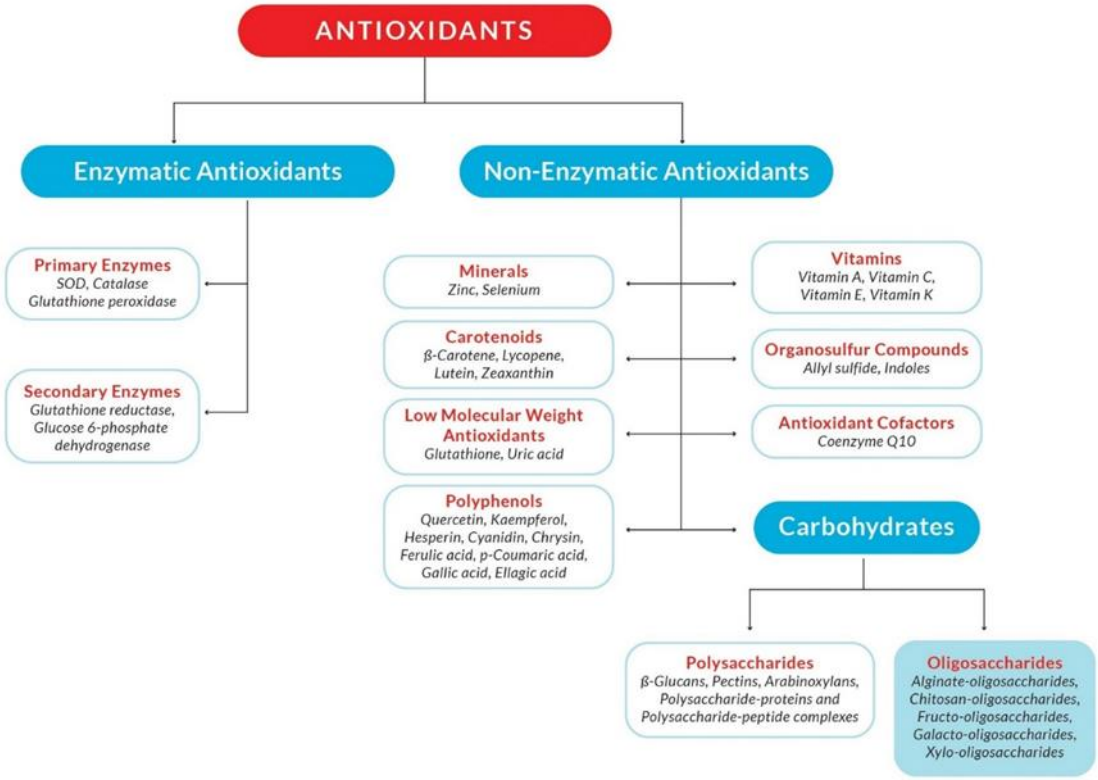

Figure 1. Classes of natural antioxidant molecules.

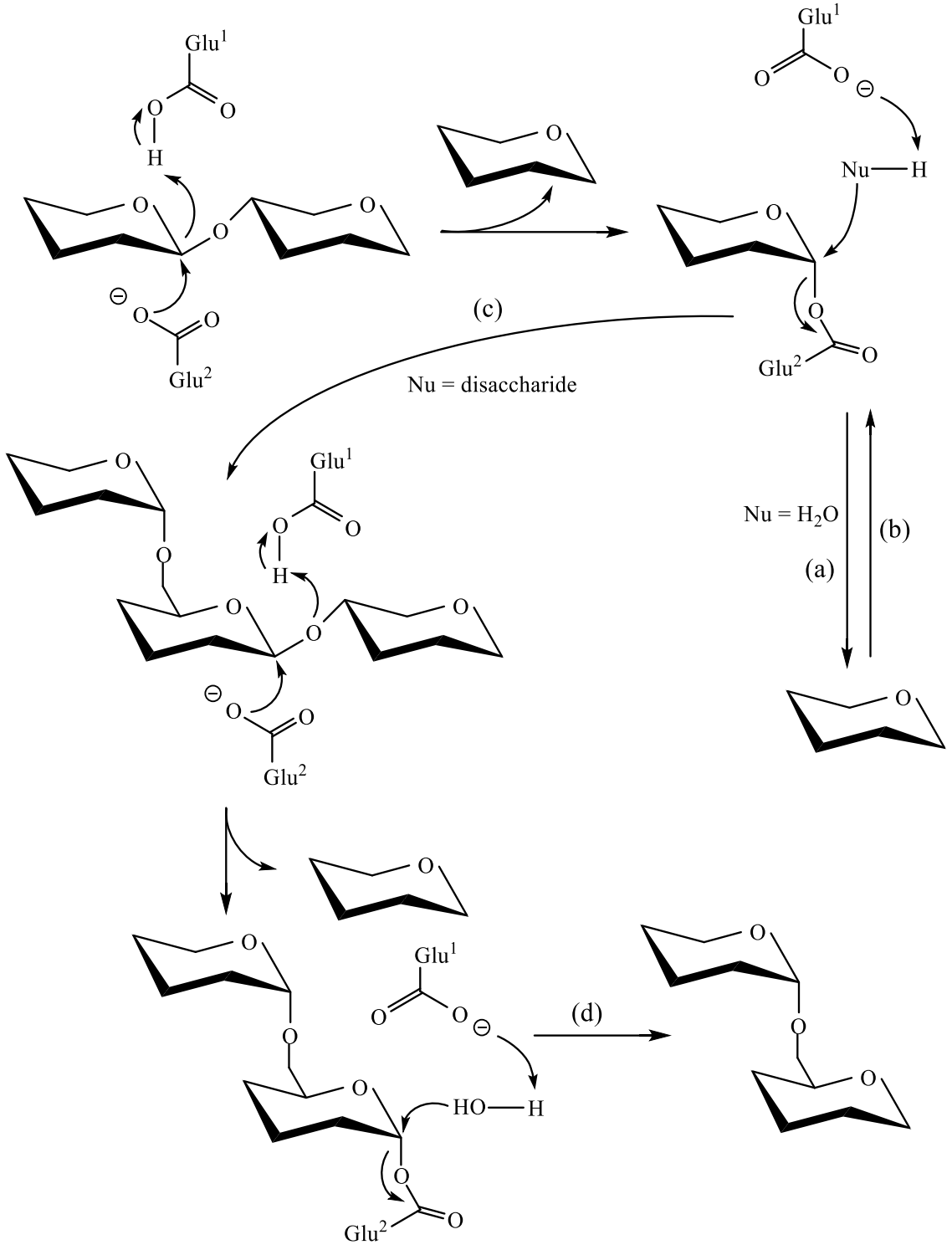

Figure 2. Generalized mechanism of a transglycosylase. (a) Hydrolysis; (b) Reverse hydrolysis; (c) transglycosylation; (d) secondary hydrolysis; Glu^1, Glu^2: glutamate; Nu: nucleophile.

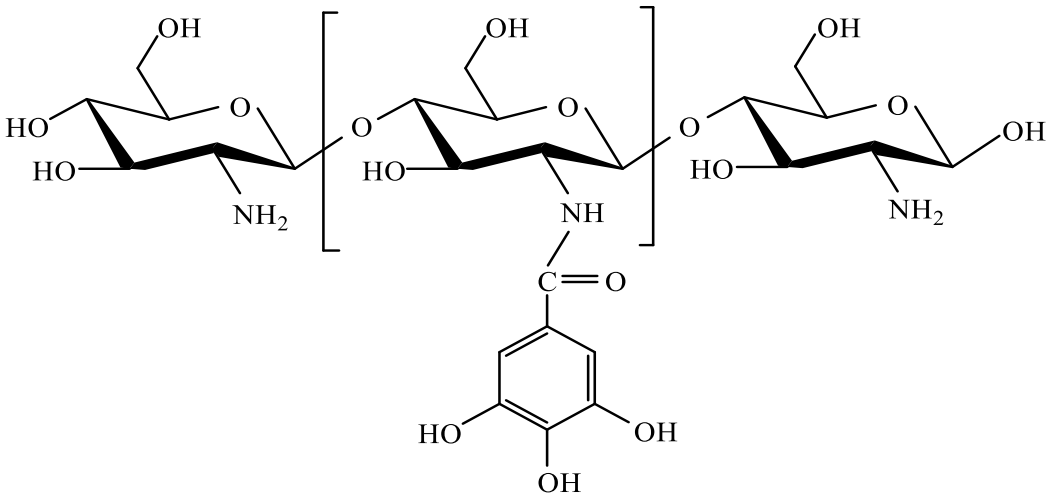

Figure 3. Galloyl-chitooligossacharide structure.

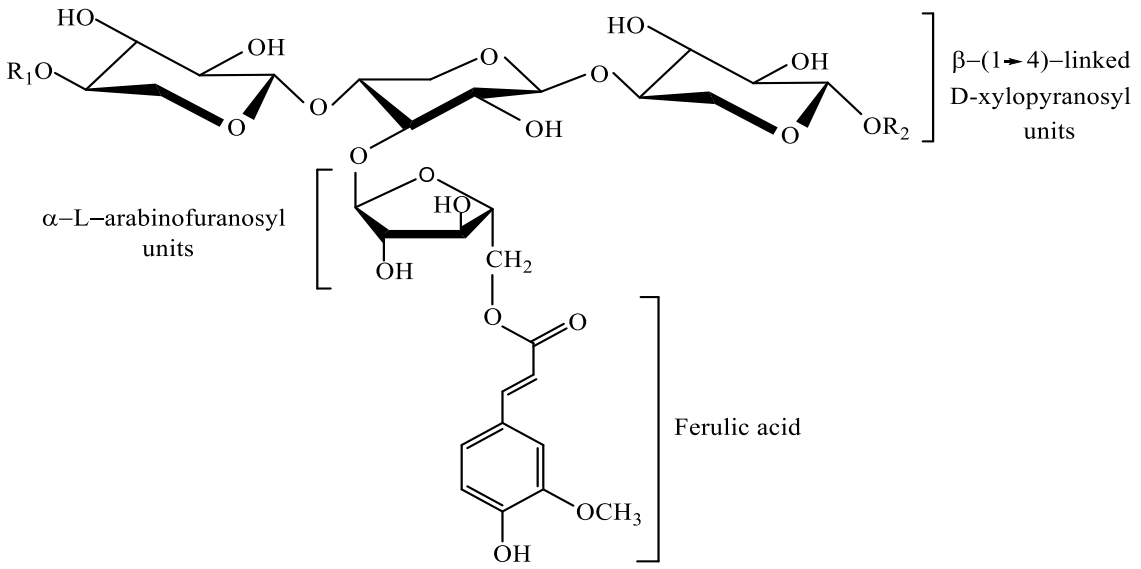

Figure 4. Feruloyl xylooligosaccharide structure.

Valorization of peach palm (*Bactris gasipaes* Kunth) waste: production of antioxidant xylooligosaccharides

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Abstract

In Brazil, the production and consumption of palm heart, especially from the *Bactris gasipaes* Kunth, generates a large number of lignocellulosic by-products. This study reports the obtainment of xylooligosaccharides (XOS) from xylans extracted from these residues. Xylans from peach palm waste (inner sheath and peel) were extracted using a mild alkali treatment with recovery yields of 82% and 80%, respectively. XOS were obtained through enzymatic hydrolysis employing a commercial xylanase with yields from xylan inner sheath and xylan peel of 50.1% and 48.8%, respectively. The antioxidant potential of XOS was measured employing five of the most commonly used procedures. In overall terms, the XOS from the xylans of peach palm wastes showed higher antioxidant capacity than the XOS obtained from the commercial xylans. The chemical structures of the XOS were determined by mass spectrometry (ESI-MS). The ESI-MS spectra suggest that XOS with grouped xylose or arabinose units ranging from 2 to 5 (differing by 132 Da) and as sodium adduct ions $[M + Na]^+$ in the range of 100-1000 *m/z*. These results indicate that peach palm wastes can be explored to XOS production, which could be applied as natural antioxidants in functional food and pharmaceutical preparations.

Keywords: peach palm wastes; xylan extraction; xylanase; xylooligosaccharide; antioxidant

Statement of Novelty

The production and consumption of palm heart (*palmito*) from the *Bactris gasipaes* (*pupunha*), generates large amount of by-products. This study reports for the first time the obtainment of antioxidant xylooligosaccharides (XOS) from xylans extracted from peach palm wastes. Chemical structures were determined and antioxidant activities were quantified. Due to their antioxidant properties, the XOS of the peach palm waste can be regarded as possessing potential for being exploited in the preparation of value-added products in both food and pharmaceutical industries. These applications, besides being linked to the bio-based economy, would certainly fit remarkably well into the biocircular economy concept. In addition, they certainly present potential of benefiting in a significant way native communities living in rain forest regions.

Introduction

Agro-industrial wastes are usually characterized by a high amount of lignocellulosic material, i.e, cellulose, hemicellulose (especially xylan), and lignin. The sources include, for example, wastes from agricultural crops that were primarily used for the extraction of starch and other food sources [1]. Forest and pulping waste products, however, are not less important in this context [2,3]

In Brazil, the production and consumption of palm heart (*palmito*), especially from the *Bactris gasipaes* Kunth species (locally known as *pupunha*), generates a large amount of by-products [4]. The State of São Paulo is the main Brazilian producer of *pupunha* palm, with about 30,000 hectares of culture, mainly in the Ribeira Valley, where there are 20 million cultivated plants [5]. Approximately 37% of the volume of all the plant matter that arrives at the industry ends as waste (sheath fibers). The latter is generated during the processing of palm hearts, being almost always used to produce animal feed or agricultural fertilizer [6]. The culture of pupunha produces between 54 and 82 tons of green mass (MV**)** per hectare, consisting of 34.2% cellulose, 21.3% hemicellulose and 19.5% lignin [7]. However, the current utilization of *B. gasipaes* palm heart wastes have little economic significance. Such biomaterials could be better exploited as sources of high-value molecules, e.g. carotenoids [8, 9] and insoluble dietary fibers [4,10], thus promoting a biocircular economy [11]

The increasing global concern about environmental issues has boosted the production of chemicals derived from sustainable resources. Within this context, the lignocellulosic biomass has gained attention as a source of several classes of compounds, obtainable by means of thermochemical and biochemical routes [12]. Lately, the growing interest in functional foods has made xylooligosaccharides a hot topic within the scientific community due to their physiological beneficial effects [13].

As the name indicates, xylooligosaccharides (XOS) are composed by xylose units (from 2 to 20) in β (1,4)-linkage. They are mostly xylobioses, xylotrioses, and xylotetraoses, which occur naturally in fruits, bamboo shoots, milk, vegetables, and honey [14]. Xylans are generally branched structures which, in addition to xylose, contain arabinose (in furanose form), uronic acid (glucopyranosidic form) or its 4-*O*-methyl derivative (2- or 3-acetyl or phenolic substituents). Branched forms, with diverse biological properties, are ubiquitous [2, 13]. XOS reach the large intestine without previous transformation in the stomach because the human body does not have the enzymes capable of hydrolyzing the β-linkages. XOS are, thus, prebiotics, which can be transformed by the colonic microbiota [15]. Besides improving the modulation of the colonic microbiota, especially bifidobacteria and lactobacilli, exerting positive effects on

bowel's function, these sugars have been shown to exert several health benefits, such as improvement in calcium absorption, protection against cardiovascular diseases, decreased risk of colon cancer, reduction of the risk of diabetes mellitus, reduction of hypercholesterolemia, immunological action, besides antioxidant, anti-inflammatory and antiallergenic effects [16].

XOS can be produced from xylan-rich lignocellulosic materials by autohydrolysis or chemical and enzymatic hydrolysis. However, the enzymatic conversion of xylan into XOS is the preferred route in the food industries, because in contrast to autohydrolysis and chemical treatment methods, the former procedure avoids the formation of undesirable by-products (toxics such as furfural) or high amounts of monosaccharides, and does not require high-pressure or high-temperature equipment [14,15,16].

Xylanases are the enzymes that hydrolyze the β-1,4 glycosidic linkages of xylan for the production of XOS. In nature, xylanolytic enzyme systems consist of endoxylanases (1,4-β-D-xylan xylohydrolases, EC 3.2.1.8), β-D-xylosidases (1,4-β-xyloside xylanohydrolases, EC 3.2.1.37) plus debranching enzymes (esterases). An efficient XOS production is only possible when β-xylosidase activity is absent or at least very low in order to avoid the production of an excess of free D-xylose. The latter is said to exert inhibitory effects in the production system [17].

Therefore, considering the need of adding value to the residues generated in the processing of the palm heart and the growing interest in the production of XOS with health beneficial properties and produced from cheap sources, the present work focuses on the extraction of xylan from peach palm heart waste and its enzymatic conversion into XOS. Furthermore, efforts have been made in order to evaluate their antioxidant and structural features. For comparative purposes, enzymatic hydrolysis of beechwood, birchwood, and oat spelt xylans were conducted in parallel. To the best of our knowledge, the peach palm wastes have not yet been exploited for such purposes.

Materials and methods

Materials

Peach palm heart (*Bactris gasipaes*) wastes, namely inner sheath and peel, were kindly donated by Embrapa Florestas, Colombo, Parana, Brazil. Purified xylanase (EC 3.2.1.8) from *Aspergillus oryzae* (X2753), beechwood xylan, birchwood xylan, oat spelt xylan and xylose standards, as well as 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic

acid (Trolox), Folin–Ciocalteu, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), orcinol, 3,5-dinitrosalicylic acid (DNS) and fluorescein reagents were obtained from Sigma-Aldrich (USA). Xylooligosaccharides (X2-X6) was purchased from Megazyme (Ireland). Water was treated in a Milli-Q water purification system (Biohuman Power I Lab Water Purifier System, Scholar UV).

Preparation and characterization of peach palm wastes (inner sheath and peel)

The materials were dried in the sunlight, milled to give a particle size of 2–3 mm thickness and used as raw materials in this study. The materials were evaluated using the technique of acid detergent fiber (ADF) to obtain the percentages of cellulose, hemicellulose and lignin, and fiber neutral detergent (NDF) for the percentage of lignocellulose [18]. The average composition of peach palm-inner sheath was 34.2 ± 4% cellulose, 19 ± 2% hemicelluloses, and 23 ± 2% lignin. The average composition of peach palm-peel was 36.0 ± 4% cellulose, 20 ± 4% hemicelluloses, and 20 ± 3% lignin.

Obtainment of xylan from peach palm wastes

The extraction of the xylan from peach palm wastes was carried out as described earlier by Samanta et al. [19], with a few modifications. The xylans were extracted in a 2 mol L^{-1} NaOH solution for 5 h at 25 °C with continuous stirring at 126 rpm. The extracts were filtered and neutralized (pH 6.0) with glacial acetic acid, followed by precipitation during an overnight period (14–16 h) with three volumes of cold ethanol. Subsequently, the precipitated xylans were centrifuged at 2544*g* (Jouan BR4i, SWM 180.5 rotor) for 15 min at 4 °C, washed twice with ethanol and once with acetone, being centrifuged after each wash and finally re-suspended in water. The acetone was evaporated in a forced air oven. Finally, the xylans were frozen, lyophilized for 48 h, and stored at room temperature for further analysis.

The following equation was used to calculate the true yield (%) of xylan:

$$\text{True yield xylan (\%)} = \frac{\text{Dry weight of extracted xylan (g)}}{\text{Weight of the sample (g)}} \times 100 \quad (1)$$

The percentage of xylans was calculated based on the original inner sheath and peel samples.

Characterization of extracted xylan from peach palm wastes

Peach palm heart wastes (inner sheath and peel) as well as their xylan fractions were characterized using Fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). FTIR spectra were determined in an IRPRESTIGE-21 Shimadzu spectrometer

with frequency range of 400–4000 cm^{-1}. Samples were prepared using KBr (2%, w/w). The thermal behavior of the biomass samples was evaluated by thermogravimetric analysis (TGA) using a Shimadzu DTG60/60H analyzer, under inert (N_2-99.995%) flow (50 mL min^{-1}). The analysis conditions were: heating rate = 10 °C min^{-1} up to 35 °C holding for 60 min and then a heating rate = 5 °C min^{-1} up to 700 °C holding for 30 min. Morphology of the samples was examined under scanning electron microscopy (SEM) using a JEOL JSM-6390LV scanning electron microscope with 1000× magnification.

Production of xylooligosaccharides

The peach palm wastes xylans (from inner sheath and peel) and three commercial xylans (from beechwood, birchwood xylan, and oat spelt) were subjected to enzymatic hydrolysis as described by Kiran et al. [20] with a few alterations. The enzyme was a commercially available endoxylanase from *A. oryzae* (Sigma-Aldrich, USA). A volume of 25 mL of a 2% (w/v) xylan suspension in sodium citrate buffer pH 5.3 was added in a 125 mL-Erlenmeyer flask. The hydrolysis was started by the addition of xylanase (30 U/g substrate). The reaction was carried out in a shaking incubator maintained at a temperature of 40 °C with a shaking speed of 110 rpm for 24 and 48 h. The samples were heated to 100 °C for 5 min to stop the hydrolysis and the mixtures were centrifuged for 5 min at 1800*g*. Unhydrolysed xylans were separated from XOS by step precipitation with three volumes of ethanol. For this purpose, 75 mL of ethanol were added to the materials and after 1 h, the samples were centrifuged at 1800*g* for 10 min. The resulting supernatants were collected and ethanol was eliminated by evaporation. Finally, the supernatants were lyophilized and maintained at -20 °C until use.

Quantification of XOS

The XOS yield was computed from both gravimetric data and the reducing sugar contents. Equation 2 was used to determine the xylooligosaccharides (w/w) yield based on measurement of dry weight of XOS:

$$\text{XOS yield}\,(\%) = \frac{\text{Dry weight of XOS mixture (g)}}{\text{Dry weight of xylan (g)}} \times 100 \quad (2)$$

XOS production was also evaluated by quantification of reducing sugars present in soluble material obtained after xylan hydrolysis by the dinitrosalicylic acid (DNS) method [21]. For the assay, 25 µL of hydrolysed sample and 25 µL of DNS reagent were added in a tube and the reaction mixtures were incubated for 5 min at 100 °C. After addition of distilled water (250 µL), the absorbance at 540 nm was

measured in spectrophotometer (UV-1800, Shimadzu, Japan). D-Xylose was used as the standard to construct the calibration curve.

Thin layer chromatography of XOS

The XOS were analyzed by thin layer chromatography (TLC) according to the protocol of Valls et al. [22], with a few alterations. Aliquots (2 µL) of each xylan hydrolysis products were applied on a Silica Gel G/UV plate (Macherey-Nagel, Germany), which constituted the solid phase. Xylose at 10 mg mL^{-1} was employed as the reference standard. The solvent system used was a mixture of chloroform, acetic acid and H_2O in a 6:7:1 ratio, respectively. For the saturation for 10 min of the chromatographic chamber with vapors from the constituents of the mobile phase, the inner sidewalls of the chamber were covered with filter paper. The chromatography, which was repeated twice, was run in the ascending mode. The silica gel plate was dried after each run and finally sprayed with a solution containing 0.2% (w/v) orcinol in sulfuric acid/ethanol (5:95, v/v). Finally, the bands corresponding to the different XOS were detected after plate heating in the oven at 100 °C during 5 min.

Antioxidant activity evaluation of XOS

Five methods were used to evaluate the antioxidant activity of XOS: Folin-Ciocalteu method, FRAP (ferric ion reducing antioxidant power) method, ORAC (oxygen radical absorbance capacity**)** method, scavenging of hydroxyl radicals method, and DPPH (2,2-diphenyl-1-picrylhydrazyl) method.

The traditional Folin-Ciocalteu method was used for assaying the total phenolic content (TPC) [23]. For constructing the standard curve various gallic acid concentrations were reacted in the same medium used for the XOS ($R^2 = 0.99$). Gallic acid was the reference for expressing the data (µg gallic acid equivalents (GAE)/mg of XOS).

The FRAP method was used as described previously [24]. Briefly, increases in absorbance at 595 nm were monitored to assess the formation of a colored $[Fe^{(III)}(TPTZ)_2]^{3+}$ complex. Freshly prepared FRAP reagent at 37 ºC (900 µL) was mixed with 90 µL of distilled water and 30 µL of each sample. Distilled water (120 µL) was used as blank. The mixture was heated to 37 °C during 30 min. The FRAP reagent was composed of 0.833 mmol L^{-1} TPTZ, 3.33 mmol L^{-1} HCl, 1.66 mmol L^{-1} $FeCl_3 \cdot 6H_2O$ and 0.2 mol L^{-1} acetate buffer, pH 3.6. The results were given as µmol Trolox equivalents (TE)/mg XOS.

The ORAC method was performed according to Dávalos et al. [25] with modifications. The reaction medium was buffered with 75 mmol L^{-1} phosphate (pH 7.4). The end volume was 200 µL. The additions were 25 µL of sample and 150 µL of fluorescein solution for a final concentration of 163 nmol L^{-1}. Preincubation was done for a period of 15 min at 37 °C with subsequent addition of the AAPH solution (25 µL). Fluorescence was read at 2 min intervals in a microplate reader during a total period of 70 min. The excitation and emission wavelengths were 485 nm and 520 nm, respectively. The blank used in each assay was phosphate buffer in place of the XOS solution. As a first step the fluorescence *versus* time curves were normalized by using the corresponding blank curve for calculating the normalization factor (= $fluorescence_{blank,t=0}/fluorescence_{sample,t=0}$). After normalization the area under this curve (AUC) was calculated using the relationship:

$$AUC = 1 + \sum_{i=1}^{i=70} \frac{f_i}{f_0} \tag{3}$$

In equation (3) f_0 stands for the initial fluorescence reading at 0 min and f_i represents the fluorescence reading at time i. The AUC of the corresponding blank was subtracted from the AUC of each sample. The latter represent, thus, net values. For all samples regression equations relating the net AUC and the antioxidant concentration were computed. ORAC values were computed as Trolox equivalents (µmol TE/mg XOS) based on a standard curve ($R^2 = 0.99$).

For measuring the hydroxyl radical scavenging activity the method described by Mu et al. [26] was used with a few modifications. The method was adapted to a microplate with additions of 20 µL of salicylic acid-ethanol solution (9.1 mmol L^{-1}), 20 µL of sample solution at different concentrations, 20 µL of $FeSO_4$ solution (9.1 mmol L^{-1}) and 120 µL of distilled water. The reaction was initiated by the addition of 120 µL of H_2O_2 (8.8 mmol L^{-1}) to the mixture and the absorbance at 510 nm was read in a microplate reader. Distilled water instead of H_2O_2 was used for the control, while distilled water instead of the sample was used for the blank. The results were expressed as the 50% efficient concentration (IC_{50}), corresponding to the concentration of substrate tested that inhibits 50% of radical absorption [27]. The parameter was obtained by interpolation in a graph of percent hydroxyl radical scavenging activity versus the lyophilized XOS concentration. The following equation was used in the calculations (where A is the absorbance):

$$\text{Hydroxyl radical scavenging activity}\,(\%) = \left[1 - \left(\frac{A_{sample} - A_{blank}}{A_{control}}\right)\right] \times 100 \tag{4}$$

Finally, the DPPH bleaching was measured according to the methodology of Thaipong et al. [28] with a few alterations. A 4.36 mg per 100 mL methanolic solution of DPPH, with an absorbance of 1.1 at 515 nm, was used as the working solution. Aqueous XOS solutions at concentrations up to 5 mg mL^{-1} were used. The XOS extracts (75 µL) were added to 1425 µL of the DPPH solution. After 1 hour the mixtures were centrifuged at 1308*g* for 5 min at 4 °C. The decrease in absorbance at 515 nm was measured against a blank containing water. The results were expressed as EC_{50} values, which were obtained by graphical interpolation in a graph in which the percentage of DPPH radical scavenging was represented against the concentration of the lyophilized XOS. Calculations were done by means of equation 5, where A is absorbance:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{A_{sample}}{A_{control}}\right) \times 100 \quad (5)$$

Mass spectrometry

The xylooligosaccharides were analyzed through direct infusion using a MICROTOF-Q II mass spectrometer (Bruker, Germany) equipped with an electrospray ion source (ESI-TOF MS) operating in positive ion mode. The selected mass range for each spectrum was 100-1000 *m/z*. For the electrospray ion source, the spray voltage was set at 4500 V and the endplate offset was -500 V. The nebulizer gas flow was 0.6 bar. The drying gas was argon 5.0 at a flow rate of 4.0 L min^{-1} at 200 ºC. Data acquisition and processing were performed using Data Analysis 4.2 software (Bruker).

Statistical analysis

The data were submitted to ANOVA with *post hoc* Student–Newman–Keuls testing. P values ≤ 0.05 were adopted for significance. The error parameters presented in tables are standard errors of the means. This treatment was carried out using the GraphPad Prism software (version 8.0).

Results and discussion

Obtainment of xylan from peach palm wastes

Xylans from peach palm wastes, inner sheath and peel, were obtained by alkaline extraction. A total of 9.51 g and 12.78 g per 100 g of starting material, respectively, were obtained. If one assumes that the inner sheath and the peel contain, respectively, 11.6% and 15.9% of xylan [10], this would correspond to recoveries of 82 and 80%. In addition to the intrinsic characteristics of the biomass, the efficiency of the xylan alkaline extraction depends considerably on the operating parameters such as temperature, concentration of NaOH, and treatment time [29]. Xylans from sugar cane bagasse [30], pigeon pea (*Cajanus cajan*) stalks [31], *Sehima nervosum* grass [32], and brown cocconut husk [33], have already been obtained through this type of extraction with high recovery yields.

Characterization of extracted xylan from peach palm wastes

The thermogravimetric behavior of biomasses is frequently divided into three regions: the hemicellulose zone (245–290 °C), the cellulose zone (290–350 °C) and the lignin zone (350–500 °C) [32,33,34]. As presented in Table 1 and Figure S1 (supplementary material), commercial xylans do not present any temperature peak at the lignin zone, as expected. On the other hand, raw peach palm shell presented the largest mass loss due the higher lignin content. On the other hand, the temperature peak corresponding to the lignin thermal decomposition disappeared from the treated biomass, indicating that the alkaline extraction was very efficient in that lignin was no longer present.

Figure 1 shows the visual appearance and morphologies of the original fibers and corresponding xylans. Similar to other plant fibers, the raw peach palm (inner sheath and shell) had a fibrous surface and was regularly arranged (Figure 1B and 1F). The fiber bundles were loosened after NaOH treatment due to the degradation of the lignin molecules from the inner parts of the fiber, and the surface presented high roughness after treatment due to the lignin removal (Figure 1D and 1H).

FT-IR spectroscopic analysis was also used to determine the structural characteristics of palm peach xylans. Figure 2 shows the spectra of the xylans obtained from palm peach waste (inner sheath and peel) compared with the spectra of the commercial birch wood xylan. They contained an identical set of transmission bands that differed, however, in intensity. A polymeric chain consisting of pure xylopyranose units should not have noticeable absorption at 1800–1500 cm^{-1} [35]. Therefore, the absorption in this region

was due to vibrations of a different type of substituent in the main chain and (or) the xylan side chains. The FTIR spectrum of extracted xylan displayed a distinct hydroxyl (OH) group stretching vibration peak at 3436 cm^{-1}, which is similar to the peak obtained by OH stretching vibration for other extracted xylans [35,36]. The peak at 1643 cm^{-1} indicates water absorption as found in previous studies [37,38]. The peak at 2929 cm^{-1} is attributable to symmetric C-H stretching, corroborating a previous report [38]. The presence of glucuronic acid is revealed by symmetric and asymmetric stretching of the carboxyl group at 1415 and 1572 cm^{-1} [39]. The FTIR spectrum region between 1200 and 1000 cm^{-1} was dominated by the ring vibration overlapped by the C–O–C glycosidic vibrations and the stretching vibration of the OH side group. The presence of the arabinosyl side chains is indicated by the low-intensity shoulder at 1164 cm^{-1}, corresponding to the C–O–C vibration [40]. The peak at 1049 cm^{-1} represents the β-1,4 backbone of xylan [35].

Production and characterization of xylooligosaccharides

The XOS yields after the enzymatic processing of the xylan from the inner sheath and peel peach palm waste, calculated according to equation 2 (gravimetric analysis), were 50.1% and 48.8% respectively, after 48 h of enzymatic hydrolysis. The XOS yields of beechwood, oat spelt and birchwood xylans, which were processed in parallel, were 80.0%, 77.5% and 76.5%, respectively. It is well-known that the XOS yield depends on the origin of the xylan-rich hemicelluloses as well as on the enzyme type and enzyme loading [40]. In addition to this, water solubility of the substrate and consequent accessibility of the enzyme, may have led to a higher yield of enzymatic conversion [41].

Veenashri and Muralikrishna [42] evaluated the production of XOS obtained from water unextractable polysaccharides of rice, ragi, wheat and maize brans through the action of xylanase. The maximum XOS yield varied from 40.0% (obtained from wheat) to 3.31% (from rice bran). According to the authors, such yield discrepancies could be due to the presence of a highly substituted xylan backbone in rice bran, making it less susceptible to cleavage by xylanase. On the other hand, the less branched wheat bran xylan had a higher number of unsubstituted xylose residues, which represent easily accessible points of cleavage, thus resulting in a higher amount of XOS with relatively low degrees of polymerization.

Rashad et al. [43] found XOS yields ranging from 54.0% to 85.5% when they investigated eight distinct hydrolysed lignocellulosic materials such as saw dust, corn cobs, sugarcane bagasse, rice straw and fruit wastes, such as mango and orange peels after 72 h of incubation with a *Bacillus amyloliquifaciens*

endoxylanase. Sugarcane bagasse was also studied by Bian et al. [39] that reported a XOS yield of 31.8% upon hydrolysis with a crude xylanase secreted by *Pichia stipitis* at a concentration of 25 U g^{-1} for 12 h.

Table 2 shows the amount of reducing sugars found after 48 h of hydrolysis of the xylans from peach palm wastes as well as the amounts of reducing sugars produced by the hydrolysis of beechwood, oat spelt and birchwood xylans. These results are in agreement with the XOS yields calculated on gravimetric grounds, as more reducing sugars were produced from beechwood, oat spelt xylan and birchwood xylans than from peach palm waste xylans.

Shi et al. [44] evaluated the yield of reducing sugars after 12 h enzymatic hydrolysis of the commercial xylans from beechwood, birchwood and oat spelt using an endoxylanase (XynBE18) secreted by *Paenibacillus* sp and found values in D-xylose equivalents equal to 9.96±0.17, 4.66±0.10 and 2.20±0.09 mmol L^{-1}, respectively. In addition, the authors reported synergistic effects on the degradation of all xylans when the endoxylanase XynBE18 and the α-L-arabinofuranosidases (Abf43A or Abf43B) were incubated in association. When the xylans were incubated sequentially, first with the α-L-arabinofuranosidase followed by incubation with the endoxylanase, the same synergistic effect were noticed.

Thin layer chromatography of XOS

The xylan hydrolysis products were analyzed by TLC (Fig. 3). Similar patterns of oligomers, i.e., XOS with varying degrees of polymerization, were obtained, being the endoxylanase capable of producing XOS with degrees of polymerization (DP) > 3, as shown in Figs. 3A and 3B.

High added value XOS could be clearly observed for all hydrolysed xylans, likely xylobiose, xylotriose, xylopentaose and xylohexaose. The occurrence of these XOS and only trace amounts of xylose as the final product confirms the endo-xylanolytic activity of the xylanase expressed in *Aspergillus oryzae*. From the intensity of the corresponding stains, the main products of xylans hydrolysis were probably xylobiose and xylotriose, and the trace amount of xylose that was detected can possibly result from xylotriose and xylobiose hydrolysis [43,44]. A trace amount, probably of xylotetraose, was also detected in all samples. In a previous report, the hydrolytic profiles of beechwood xylan and birchwood xylan were found to be similar because they are both *O*-acetyl-4-*O*-methylglucurono hardwood xylans [45].

Antioxidant activities

There is now widespread acceptance that patients suffering from several diseases (e.g., rheumatoid arthrits, cardiovascular diseases, etc.) present high levels of reactive oxygen species. Actually, in at least some cases, the disease can even be triggered by an unbalanced redox state [46,47]. For this reason, and in line with previous reports on natural polymeric compounds isolated from various species [48,49,50], special efforts were dedicated in this work to the characterization and quantification of the antioxidant effects of the XOS that were isolated in the present work. To this purpose five methods were used: TPC with the Folin-Ciocalteu reagent, FRAP, hydroxyl radical scavenging activity, ORAC and DPPH assays (Table 3).

The values of TPC ranged from 0.89±0.02 to 4.93±0.3 μg GAE·mg^{-1} of XOS, with a significantly higher value for the XOS from the peach palm waste-peel xylan. It is important to note that, at least in part, the TPC values are likely to reflect a significant contribution of lignin monomers that precipitated with the xylan during the isolation procedure [50]. Rashad et al. [43] quantified the TPC of XOS produced from different agricultural residues including corn cobs, wheat bran, sugarcane bagasse, rice straw, water hyacinth, mango peels, saw dust and orange peels. The highest values of TPC were found for the mixtures of XOS from orange peel and mango peel. The Folin–Ciocalteau colorimetric method was also used by Mandelli et al. [51] for quantifying the TPC in commercial wheat arabinoxylan, *in natura* sugarcane bagasse, steam-exploded sugarcane bagasse and chemically treated sugarcane bagasse after hydrolysis with XynZ from *Clostridium thermocellum*.

Table 3 shows the antioxidant activities of XOS as determined by the FRAP assay. The values ranged from 9.01±0.33 to 16.39±1.07 μmol TE·mg^{-1}. The XOS from peach palm waste-peel xylan showed a FRAP value equal to the values presented by the XOS from birchwood xylan and from oat spelt xylan. This assay has already been used to evaluate the antioxidant activities of the XOS derived from rice, corn, wheat, and ragi [42]. The authors reported good antioxidant activity especially for XOS mixtures from ragi, which was concentration-dependent unlike the activities of the other samples, which even at high concentrations, did not present such behavior.

The antioxidant abilities of XOS were also determined by means of the hydroxyl radical scavenging activity and expressed as the concentration required to obtain 50% inhibition (IC_{50}) (Table 3). High abilities correspond, thus, to low IC_{50} values. Although there was a tendency for lower IC_{50} values for the XOS from birchwood xylan and oat spelt (3.46 ± 0.57), significant statistical differences were not observed among all XOS. Hydroxyl radical scavenging activities among XOS have been frequently

reported. For example, this method was used in the evaluation of antioxidant activities of XOS from beechwood [52]. In this work, the XOS were obtained by enzymatic hydrolysis at 80 °C using a cocktail of xylanase (XynB) and α-glucuronidase or a single XynB from *Thermotoga maritime.* More recently, the same method was used for evaluating the antioxidant activity of XOS obtained from a *Moso bamboo* prehydrolyzate after 12 h of enzymatic hydrolysis using an endo-β-1-4-xylanase [53].

The fourth method used in this work to evaluate the antioxidant activities of XOS was the ORAC assay (Table 3). The highest antioxidant activity was found in the XOS from peach palm waste-peel xylan (44.15 ± 4.33 μmol TE·mg^{-1}), which was significantly higher than that of all other XOS (Table 3). Up to the present time, no other measurements of antioxidant activity of XOS with the ORAC method are available in the specialized literature.

Finally, Table 3 presents data where the antioxidant activities of XOS were evaluated by using the DPPH scavenging method. The absorbance decrease was monitored at 515 nm. Extent of the decrease was characterized by a clear dose-dependent behaviour. The XOS from peach palm waste-peel xylan displayed the highest ability towards the other samples in all studied concentrations, followed by XOS from oat spelt and XOS from the inner sheath xylan of peach palm waste. The XOS from beechwood and birchwood xylans did not exhibit antioxidant activity at the lowest concentration range. The behaviour of the XOS samples as DPPH radical scavengers was similar to that in the TPC and ORAC assays described previously. The DPPH assay has been very often used to quantify free radical scavenging activity of XOS. For instance, it was used to assess the antioxidant activity of the XOS obtained after 12 h of enzymatic hydrolysis of sugarcane bagasse using a crude xylanase from *Pichia stipites* [39]. Jagtap et al. [54] have also used this method to evaluate the antioxidant activity of XOS from wheat husk obtained after 12 h of enzymatic hydrolysis with a crude xylanase produced by *Aspergillus fumigatus* R1. Likewise, the method was used to estimate the antioxidant potential of XOS obtained from rice, corn, wheat, and ragi [42]

The antioxidant activities of oligosaccharides including XOS may vary considerably by a series of reasons such as the method of depolymerization, the degree of polymerization, the solubility, the nature of the monosaccharide constituents, the glycosidic linkages of the side chains and the molecular weight]. Additional important factors are the presence of phenolic groups (e.g. ferulic acid) and carboxylic groups (belonging to uronic acid, for example), which are well known for their antioxidant reactivity [42]. For these reasons, it is important to use different methods for an accurate quantification of the antioxidant activity.

Mass spectrometry

Mass spectrometry was used to evaluate the structures of XOS generated by enzymatic hydrolysis of the various xylans. The corresponding spectra show the presence of ions in the range of 100 to 1000 *m/z* (Fig. 4). The ion signals in *m/z* and the carbohydrates assigned to them with their molecular formulas and chemical structures can be seen in Table 4. The XOS were identified as presenting xylose or arabinose units ranging from 2 to 5 (differing by 132 Da) and as sodium adduct ions ($[M+ Na]^+$). This is consistent with the reports of Reis et al. [55] and Manisseri and Gudipati [56], which studied the XOS from purified olive pulp and wheat bran, respectively. It is worth mentioning that the molecular mass of xylose and arabinose is the same (150 $g.mol^{-1}$) and that they cannot be distinguished.

All studied XOS mixtures showed preponderantly the presence of an ion at *m/z* 305, followed by *m/z* 437, identified as the disaccharide xylobiose (or arabinofuranosyl-xylose) and the trisaccharide xylotriose (or arabinofuranosyl-xylotriose) (Fig. 3). These results are in accordance with TLC analysis, in which xylobiose and xylotriose are likely to be the main xylan hydrolysis products. The ion at *m/z* 437 was more abundant in XOS mixtures from peach palm waste-peel xylan and peach palm waste-peel xylan than in the XOS mixtures derived from commercial xylans. Furthermore, the spectra obtained for the XOS mixtures of beechwood and birchwood xylans were very similar, since they are xylans of *O*-acetyl-4-*O*-methylglucurone, as previously deduced from the TLC analysis.

The molecular ion signal at *m/z* 701 found in the XOS mixture from oat spelt xylan and much less abundant in the XOS mixtures from peach palm waste-inner sheath xylan, can be assigned to the pentasaccharide xylopentose or arabinofuranosyl-xylotetraose.

The XOS mixture derived from the peach palm waste-inner sheath xylan also showed ion signals at *m/z* 173, 215, 237, 259, 383, 473 and 569. The signals at *m/z* 173, 215 and 569 were identified as xylose or arabinose, acetyl-xylose and xylotetraose or arabinofuranosyl-xylotriose, respectively. However, to date we have not been able to identify the signals at *m/z* 154-155, 237, 383 and 473. The ion at *m/z* 383 appeared in the XOS ESI-MS spectrum derived from the millet seed coat obtained by Palaniappan et al. [57], but they also failed to identify it. Thus, for further clarifying the structure of the XOS obained in the present work, procedures that go beyond mass spectrometry must be employed, especially one of the various NMR (nuclear magnetic ressonance) techniques [58, 59,60].

Conclusions

Although XOS via enzymatic hydrolysis of xylans have already been obtained from different agroindustrial wastes, to the best of our knowledge, this is the first communication of an antioxidant XOS obtainment from xylans of peach palm wastes. Efforts were done to characterize the XOS chemical structures as well as to compare their antioxidant activities with those of XOS obtained from commercial xylans. In overall terms, with respect to the free radical scavenging capacity, the XOS from xylan of peach palm wastes showed higher antioxidant capacity than that of three XOS obtained from commercial xylans (birchwood, beechwood and oat spelt). Due to their antioxidant properties, the XOS of the peach palm waste can be regarded as possessing potential for being exploited in the preparation of value-added products by the food and pharmaceutical industries. These applications, besides being linked to the bio-based economy, would certainly fit remarkably well into the biocircular economy concept. In addition, they certainly present potential of benefiting in a significant way native communities living in rain forest regions.

Competing Interest

There are no interest conflicts.

Funding

This work was supported by the National Council of Scientific and Technological Development (CNPq, Proc. 404898/2016-5 and 304406/2019-8) and by the Araucaria Foundation (Proc. 03/2019). Author T.F. Vieira is a graduate scholarship fellow of the Coordination for the Improvement of Higher Education Personnel (CAPES); R.C.G. Corrêa is a research grant recipient of Cesumar Institute of Science Technology and Innovation (ICETI).

Author contributions

Conceptualization and data curation; T. F. Vieira; R. C. G. Corrêa. **Formal analysis;** T. F. Vieira; J. A. A. Garcia; **Funding acquisition;** R. M. Peralta; **Investigation; Methodology;** T. F. Vieira; J. A. A. Garcia; **Project administration;** R. M. Peralta; **Supervision;** A. Bracht; R. M. Peralta; **Validation and Visualization;** R. C. G. Correa; R. A. Peralta; R. F. Peralta Muniz Moreira; **Writing - original draft;** R. F. Peralta Muniz Moreira, R. A. Peralta, E. A. de Lima, C. V. Helm; **Writing - review & editing.** A. Bracht; R. M. Peralta

Acknowledgments

The authors thanks EMBRAPA-FLORESTAS for supplying the palm heart wastes.

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Table 1 Weight loss and temperature of weight peak loss during thermogravimetric analysis

Sample	Mass loss (%)	DTG peaks (°C)
Peach palm waste (inner sheath)	75.85	149/324/487
Peach palm waste (peel)	78.00	289/338/344
Xylan from peach palm (inner sheath)	71.34	297
Xylan from peach palm (peel)	66.80	276
Birchwood xylan	73.21	236/292/298
Beechwood xylan	67.87	237/295
Oat spelt xylan	68.06	183/254/283

Table 2 Production of reducing sugars by enzymatic hydrolysis of xylans

Xylan sources	Reducing sugars (mg/g)		
	0 h	48 h	Δ (48-0)
Peach palm (inner sheath) waste	9.36±1.40[a]	256.90±27.30[a]	247.54±[a]
Peach palm (peel) waste	24.50±2.15[b]	276.50±29.40[a]	252.00±[a]
Beechwood	33.16±2.90[c]	555.63±45.80[b]	522.47±[b]
Birchwood	16.63±1.50[d]	422.63±37.80[c]	406.00±[c]
Oat spelt	8.58±1.20[a]	539.00±39.40[b]	530.42±[b]

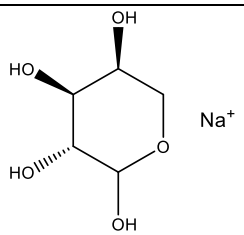
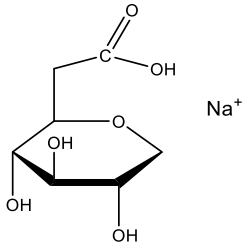
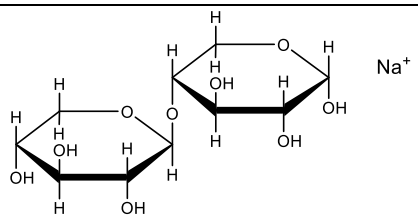
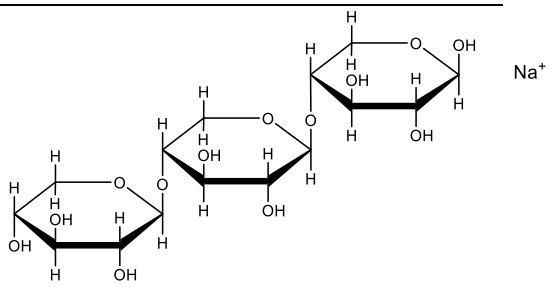
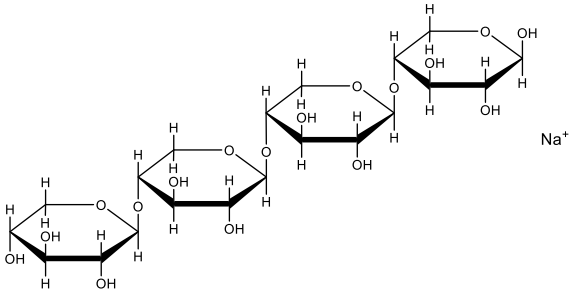
Different letters overwritten to the averages of the same column indicate a significant difference at the level of 5%.

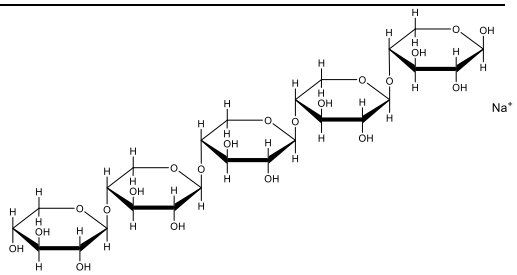
Table 3 Antioxidant activities of the XOS obtained by enzymatic hydrolysis of peach palm waste (inner sheath and peel), beechwood, birchwood, and oat spelt xylans

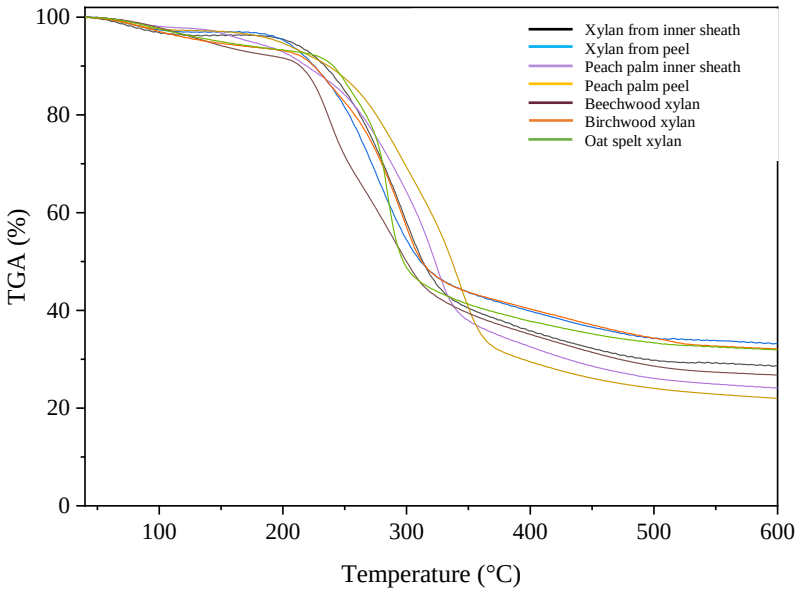
Xylan source	Antioxidant methods				
	TPC (μg GAE/mg of XOS)	FRAP (μmol TE/mg of XOS)	Hydroxyl radical scavenging activity IC_{50} values (mg/mL)	ORAC (μmols TE/mg of XOS)	DPPH IC_{50} values (mg/mL)
Peach palm (inner sheath) waste	1.21 ± 0.06^{c}	9.01 ± 0.33^{c}	4.17 ± 0.46^{a}	14.82 ± 0.70^{c}	3.88 ± 0.14^{a}
Peach palm (peel) waste	4.92 ± 0.30^{a}	14.40 ± 0.53^{b}	3.57 ± 0.46^{a}	44.14 ± 4.33^{a}	2.15 ± 0.36^{c}
Beechwood	0.94 ± 0.03^{c}	10.51 ± 1.06^{c}	3.48 ± 0.22^{a}	6.88 ± 0.75^{d}	> 5.0
Birchwood	0.89 ± 0.02^{c}	16.39 ± 1.07^{a}	3.41 ± 0.22^{a}	12.83 ± 1.50^{c}	> 5.0
Oat spelt	3.03 ± 0.07^{b}	$15.45 \pm 0.50^{a,b}$	3.46 ± 0.57^{a}	21.52 ± 2.83^{b}	3.24 ± 0.23^{b}

Different letters overwritten to the averages of the same column indicate a significant difference at the level of 5%.

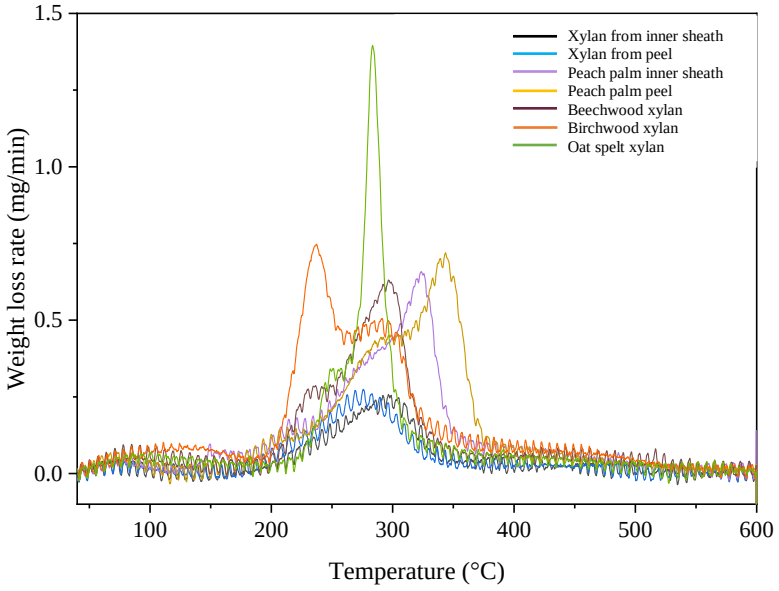
Table 4 Ion signals at various *m/z* and the carbohydrates assigned to them with their corresponding molecular formulas and chemical structures

m/z	**Carbohydrate**	**Molecular formula**	**Chemical structure**
173.0627	Xylose, arabinose	$C_5H_{10}O_5Na$	
215.0631	Acetyl-xylose	$C_7H_{12}O_6Na$	
305.1342	Xylobiose, arabinofuranosyl-xylose	$C_{10}H_{18}O_9Na$	
437.0873	Xylotriose, arabinofuranosyl-xylobiose	$C_{15}H_{26}O_{13}Na$	
569.2612	Xylotetraose, arabinofuranosyl-xylotriose	$C_{20}H_{34}O_{17}Na$	

701.3221	Xylopentaose, arabinofuranosyl-xylotetraose	$C_{25}H_{42}O_{21}Na$	



(a)



(b)

Fig. S1 TGA (a) and DTG (b) analyses of different biomasses used in this work

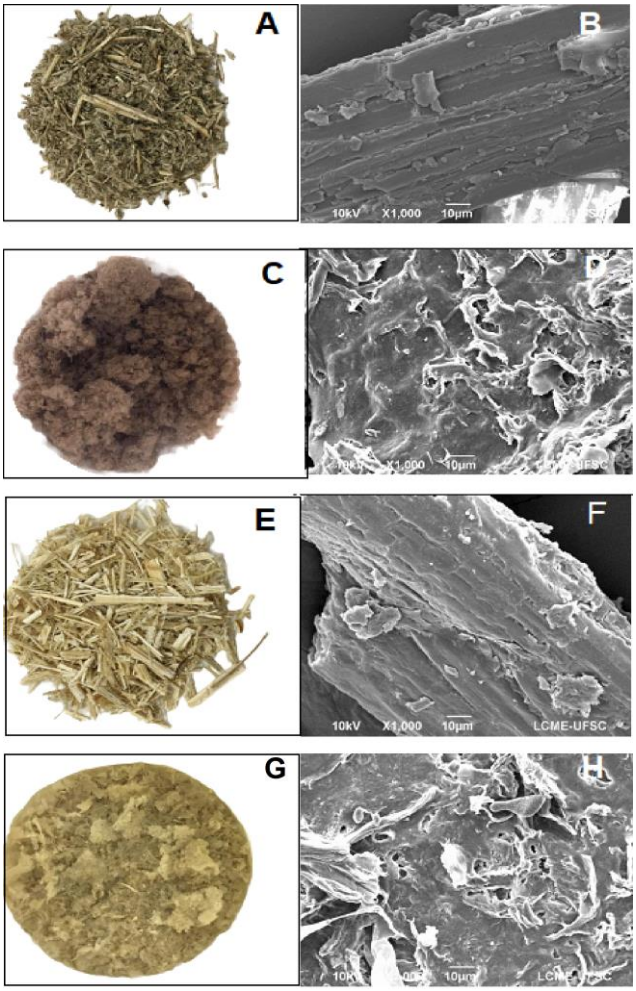


Fig. 1 Optical photographs and scanning electron microscopy of peach palm waste. A,B: raw peach palm-peel; C, D: peach palm-peel after NaOH extraction; E, F: raw peach palm-inner sheath; G, H: peach palm-inner sheath after NaOH extraction

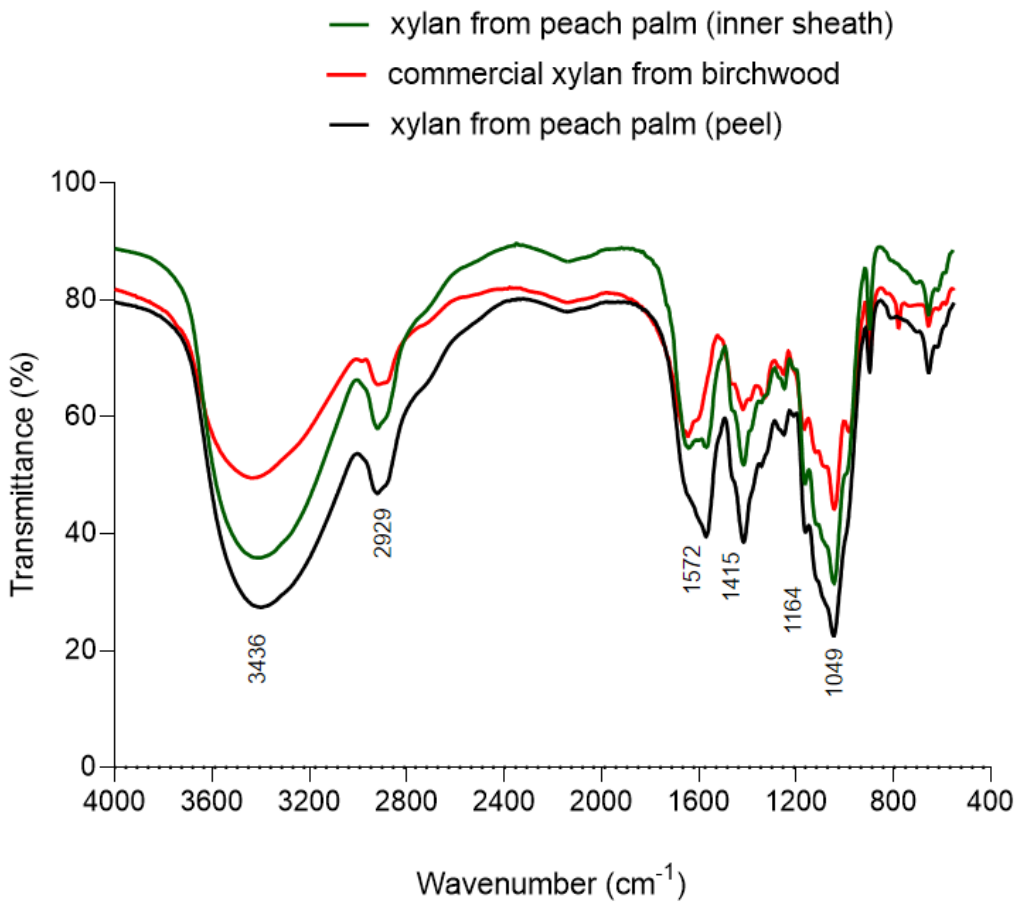


Fig. 2 FT-IR spectra the xylans used in the present work

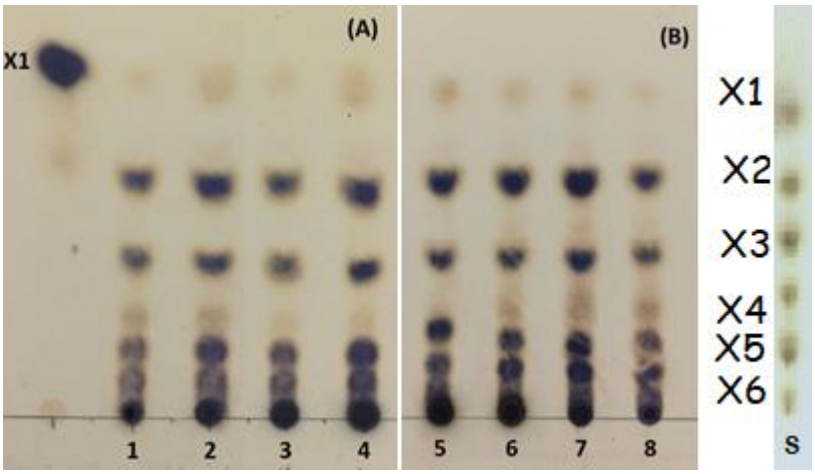


Fig. 3 TLC analysis of the XOS released after hydrolysis of xylans from peach palm waste (inner sheath and peel) (A) and commercial (B) xylans using an commercial endo-xylanase from *Aspergillus oryzae* In A: X1: xylose (10 µg/µL); Lane 1: XOS from peach palm waste-inner sheath xylan after 24 h of hydrolysis, lane 2: XOS from from peach palm waste-inner sheath xylan after 48 h of hydrolysis, lane 3: XOS from peach palm waste-peel xylan after 24 h of hydrolysis, lane 4: XOS from peach palm waste-peel xylan after 48 h of hydrolysis; (B) Lane 5: XOS from oat spelt xylan after 48 h of hydrolysis, lane 6: XOS from birchwood xylan after 48 h of hydrolysis, lane 7: XOS from beechwood xylan after 48 h of hydrolysis, lane 8: XOS from beechwood xylan after 24 h of hydrolysis. Lane S: standard XOS containing xylose (X), xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5) and xylohexaose (X6).

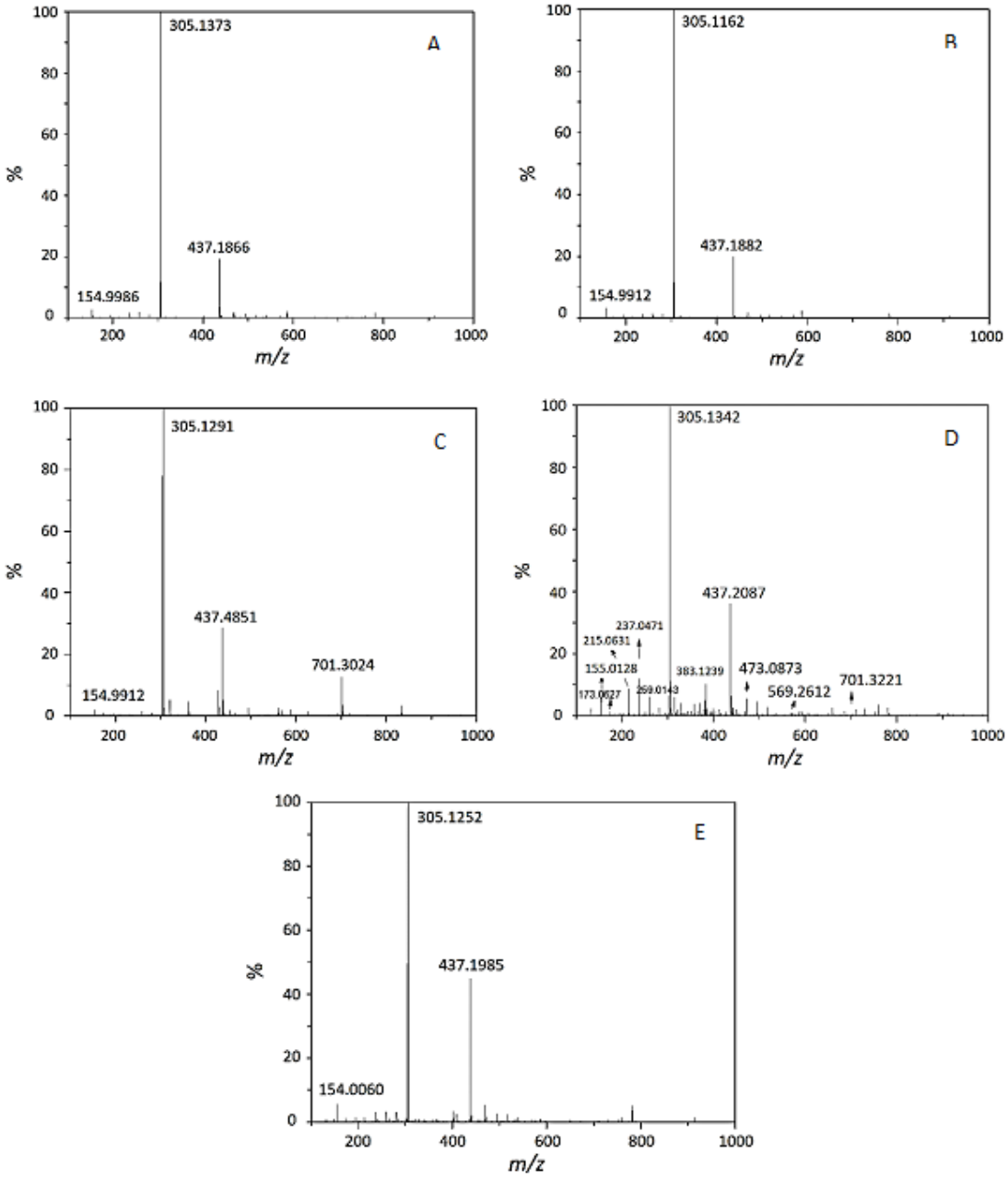


Fig. 4 ESI-MS spectra of xylooligosaccharides produced by *A. oryzae* xylanase from: (A) beechwood xylan; (B) birchwood xylan; (C) oat spelt xylan; (D) peach palm (inner sheath) waste xylan; and, (E) peach palm (peel) waste