UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

JHONATAS RODRIGUES BARBOSA

Obtenção de Extratos Polissacarídicos do Cogumelo Comestível *Pleurotus Ostreatus* com Tecnologia de Fluido Supercrítico

BELÉM-PARÁ
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Orientador: Prof. Dr. Raul Nunes de Carvalho Junior.

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Não posso provar que deus não existe, mas também não posso provar que cogumelos não poderiam estar em espaçonaves intergalácticas nos espionando.

Daniel Dennett

RESUMO

A presente proposta de dissertação buscou desenvolver um método eficiente para recuperação de extratos ricos em polissacarídeos a partir do cogumelo comestível *Pleurotus ostreatus* usando um sistema binário de água quente e CO_2 supercrítico. A dissertação foi estrutura em dois capítulos interligados. O capitulo I, é um artigo publicado de revisão bibliográfica, onde são abordados de forma clara e factual os últimos achados da literatura sobre o tema, dando ênfase ao desenvolvimento de novas técnicas de extração, potencial biológica e novas tecnologias. O capítulo II é um artigo de pesquisa, onde estão dispostos os principais achados sobre o processo de extração e caracterização de uma fração rica em polissacarídeos. Os resultados mostram que a tecnologia e o processo desenvolvidos foram eficientes para recuperação com alto rendimento de extratos ricos em polissacarídeos. Além disso, a pesquisa demostrou que os extratos são uma mistura de heteropolissacarídeos, β-glucanos, α-glucanos e oligossacarídeos. O extrato da melhor condição de extração tem alta atividade antioxidante, não possuem efeitos citotóxicos e apresentam atividade citoprotetora contra radicais livres. Assim, o método desenvolvido para recuperação de extratos ricos em polissacarídeos é eficiente, ecologicamente correto e possui potencial para implementação industrial.

Palavras-chaves: Polissacarídeos; *Pleurotus ostreatus*; Tecnologia supercrítica; Sistema binário; Citoproteção.

ABSTRACT

The present dissertation proposal sought to develop an efficient method for recovery of extracts rich in polysaccharides from the edible mushroom *Pleurotus ostreatus* using binary system with hot water and supercritical CO_2. The dissertation was structured in two interconnected chapters. Chapter I is a published article of review bibliographic, in which the latest findings of the literature on the subject are addressed clearly and factually, emphasizing the development of new extraction techniques, biological potential, and new technologies. Chapter II is a research article, where the main findings about the extraction and characterization process of a fraction rich in polysaccharides are displayed. The results show that the technology and process developed were efficient for recovery with a high yield of extracts rich in polysaccharides. In addition, research has shown that the extracts are a mixture of heteropolysaccharides, β-glucans, α-glucans, and oligosaccharides. The extract with the best extraction condition has high antioxidant activity, does not have cytotoxic effects, and has cytoprotective activity against free radicals. Thus, the method developed to recover extracts rich in polysaccharides is efficient, environmentally friendly and has potential for industrial implementation

Keywords: Polysaccharides; *Pleurotus ostreatus*; Supercritical technology; Binary system; Cytoprotection;

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SUMÁRIO

1. INTRODUÇÃO GERAL

Os cogumelos são fontes importante de compostos bioativos, em especial metabolitos primários (polissacarídeos e proteínas) e secundários (alcaloides, flavonoides e pigmentos). Em geral, estes compostos são produzidos pelos fungos através de uma complexa rede de síntese orgânica, que inicia com a obtenção de compostos simples como carbono e nitrogênio, provenientes da matéria orgânica (HALBWACHS; SIMMEL; BÄSSLER, 2016). A capacidade de reciclar e remodelar compostos simples para produção de compostos orgânicos complexos é mediada por inúmeras reações de acoplamento proteico, catalisadas por enzimas especificas (RUTHES; SMIDERLE; IACOMINI, 2015). A capacidade de produção enzimática dos cogumelos é a chave para a compreensão da especificidade na produção de tantas classes de metabolitos primários e secundários.

Os polissacarídeos são polímeros orgânicos universais, presentes em todos os organismos vivos. No entanto nos fungos estes polímeros exercem papel central na bioquímica e na biologia, sendo parte influente de toda a evolução dos fungos. Durante a síntese destes compostos, são necessárias enzimas especificas, que atuam como iniciadoras, modeladoras, remediadoras e finalizadoras de reações de gliconjugação (RUTHES; SMIDERLE; IACOMINI, 2015). Nesse cenário, a produção de polissacarídeos pelos fungos é estratégica a sua própria sobrevivência, reprodução e evolução. Os polissacarídeos, são parte relevante da parede celular dos fungos, onde atuam como camada protetora, locais avos de acoplamento molecular, sítios de interações, reações, controladores do índice de acidez e umidade (CHEN et al., 2019).

Pesquisas recentes com fungos exploraram a extração de polissacarídeos (DATTA et al., 2019; KALETA et al., 2019; LIAO; HUANG, 2019), dando ênfase, principalmente as técnicas de extração, caracterização químico, molecular, propriedades físicas e atividades biotecnológicas. Nesse contexto, os fungos do gênero *Pleurotus spp* foram estudados, diversos polissacarídeos foram descobertos, isolados, caracterizados e avaliados quanto ao seu potencial bioativos. Entre os fungos do gênero *Pleurotus spp*, a espécie *Pleurotus ostreatus*, mais conhecida por seu nome comercial, cogumelo ostra, foi relatada em pesquisas recentes como sendo fonte de polissacarídeos bioativos, em especial, com atividade antioxidante e antitumoral (KHAN et al., 2019).

Atualmente o crescente interesse por polissacarídeos de cogumelos tem chamado a atenção de empresas farmacêuticas e alimentícias. Estas empresas possuem interesse

crescente na exploração comercial destes polímeros, principalmente para formulações de produtos como fármacos antitumorais e constituintes em processos de fabricação de alimentos, para atuarem como agentes espessantes e antioxidantes (DATTA et al., 2019). O principal problema para exploração destes biopolimeros são as técnicas de extração, notadamente pouco eficientes, de alto custo e baixo aproveitamento para recuperação de polissacarídeos. Conforme é demostrado no capitulo **I**, várias técnicas de extração já foram abordadas em diversas pesquisas. Mais fica claro que o desenvolvimento de uma nova metodologia usando um conjunto de tecnologias inovadoras pode ser a chave para a consolidação de um processo eficiente de extração, ou recuperação de extratos ricos em polissacarídeos.

Com base nesta problemática, o desenvolvimento de um processo usando um sistema binário de extração, composto por água quente e dióxido de carbono supercrítico pode ser a chave para a exploração de recursos naturais ricos em polissacarídeos. A principal vantagem da tecnologia está associada ao uso de solvente verde, com baixa toxidade aos seres humanos e ao meio ambiente; condições termodinâmicas de temperatura e pressões críticas moderadas (Tc= 31 oC e Pc= 72,9 bar): e por fim, talvez a questão mais fundamental, o extrato é preservado de contaminações por solvente e do contato com o ar, que poderia ocasionar ou desencadear reações de oxidação (BADENS et al., 2018). Assim, o desenvolvimento da tecnologia e do processo poderão fornecer bases de dados suficientes para a construção de modelos e projetos para no futuro termos condições de ampliação de escala, contribuindo para a implementação de uma indústria sustentável e eficiente.

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2. OBJETIVO

2.1 Objetivo geral

O objetivo geral deste trabalho foi desenvolver um processo de obtenção de extratos polissacarídicos do cogumelo comestível *Pleurotus ostreatus* com tecnologia de fluido supercrítico e avaliar o potencial antioxidante e citoprotetor.

2.2 Objetivos específicos

- ✓ Determinar o melhor leito de extração
- ✓ Determinar as melhores condições de extração usando planejamento experimental.
- ✓ Definir a melhor condição de extração que maximizassem o rendimento global e a composição em polissacarídeos.
- ✓ Caraterização química e morfológica dos estratos polissacaridicos.
- ✓ Avaliar a atividade antioxidante dos extratos polissacaridicos.
- ✓ Avaliar a citotoxicidade e citoproteção da condição otimizada.

3. ESTRUTURA DA DISSERTAÇÃO

A dissertação de mestrado foi organizada na forma mista, contendo inicialmente um resumo e introdução geral, onde são abordados todo o conteúdo da dissertação de forma resumida e concisa. Em seguida foram organizados dois capítulos, na forma de artigos científicos, onde são abordados a revisão bibliográfica e os resultados da pesquisa, conforme pode ser observado no texto baixo, na descrição de cada capitulo.

Capitulo I: Artigo de revisão bibliográfica publicado na revista Carbohydrate Polymers no ano de 2020, com o título "**Polysaccharides of mushroom *Pleurotus spp*: New extraction techniques, biological activities and development of new Technologies**". O artigo aborda de forma ampla os últimos achados em polissacarídeos de cogumelos do gênero *Pleurotus spp*, dando ênfase as novas técnicas de extração, as bioatividades, com enfoque nos mecanismos e rotas de ação, e por fim as novas tecnologias com polissacarídeos, como produção de polissacarídeos selenizados e vacinas.

Capitulo II: Artigo de pesquisa publicado na revista Food Chemistry no ano de 2020, com o título "**Obtaining extracts rich in antioxidant polysaccharides from the edible mushroom *Pleurotus ostreatus* using binary system with hot water and supercritical CO_2**". O artigo relata o desenvolvido um método eficiente para recuperação de extratos polissacarídicos do cogumelo comestível *Pleurotus ostreatus* usando um sistema binário com água quente e CO_2 supercrítico. Todo o processo, desde o desenvolvimento do leito de extração até a etapa de recuperação de extratos polissacaridicos foi otimizada usando planejamento experimental. Por fim, os extratos polissacarídicos foram quantificados, caracterizados e analisados quanto a atividade antioxidante, citotoxicidade e citoproteção.

CAPÍTULO I

Polysaccharides of mushroom *Pleurotus spp*: New extraction techniques, biological activities and development of new Technologies

Jhonatas Rodrigues Barbosa, Maurício Madson dos Santos Freitas, Luiza Helena da Silva Martins, Raul Nunes de Carvalho Junior

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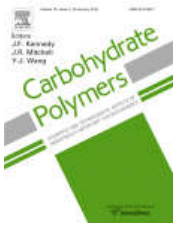

Polysaccharides of mushroom Pleurotus spp.: New extraction techniques, biological activities and development of new technologies

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Polysaccharides of mushroom *Pleurotus* spp.: New extraction techniques, biological activities and development of new technologies

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ABSTRACT

The biodiversity of mushrooms *Pleurotus* spp. is impressive due to its complexity and diversity related to the composition of chemical structures such as polysaccharides, glycoproteins and secondary metabolites such as alkaloids, flavonoids and betalains. Recent studies of polysaccharides and their structural elucidation have helped to direct research and development of technologies related to pharmacological action, production of bioactive foods and application of new, more sophisticated extraction tools. The diversity of bioactivities related to these biopolymers, their mechanisms and routes of action are constant focus of researches. The elucidation of bioactivities has helped to formulate new vaccines and targeted drugs. In this context, in terms of polysaccharides and the diversity of mushrooms *Pleurotus* spp., this review seeks to revisit the genus, making an updated approach on the recent discoveries of polysaccharides, new extraction techniques and bioactivities, emphasising on their mechanisms and routes in order to update the reader on the recent technologies related to these polymers.

1. Introtuction

In current years, a large number of publications and reviews have impacted the international scientific community demonstrating the feasibility of the use of mushroom polysaccharides as potent pharmacological agents with multivariate bioactivities (Bai et al., 2019; Cheng, Wang, He, & Wei, 2018; Kothari, Patel, & Kim, 2018; Rathore, Prasad, Kapri, Tiwari, & Sharma, 2019; Ruiz-Herrera & Ortiz-Castellanos, 2019; Sun, Zhang, & Fang, 2019). It is noteworthy that, in the context where the biological diversity of the planet is undergoing major changes, research on mushrooms has helped to elucidate the enormous potential of these organisms for the development of new technologies, drugs, food and cosmetics (Ma, Yang et al., 2018; Reis, Martins, Vasconcelos, Morales, & Ferreira, 2017).

The genus *Pleurotus* spp. is probably the best known edible mushroom genus in the world due to its gastronomic, nutritional importance and also the latest findings, which revealed potent bioactivities, such as anti-inflammatory (Taofiq et al., 2015; Yan et al., 2019b), antioxidant (Xu et al., 2016; Yan, Meng, et al., 2019), antidiabetic (Khatun, Islam, Cakilcioglu, Guler, & Chatterjee, 2015; Zhang, Zhang et al., 2018, 2018b), antitumor, (Wu, Hu, Huang, & Jiang, 2013; Zhang, Li et al., 2016, 2016b; Zhang, Yang, Jin, Yang, & Zhang, 2016) and immunostimulator (Liu et al., 2019a, 2019b; Vetvicka et al., 2019). Several mushrooms of this genus are considered true gastronomic delicacies such as the *Pleurotus* ostreatus, better known as Shimeji. This mushroom stands out from all others in the genus as it represents major advances in polysaccharide research (Bai et al., 2019; Roncero-Ramos & Delgado-Andrade, 2017) and vaccine production (Del Giudice, Rappuoli, & Didierlaurent, 2018; Sun et al., 2018).

The genus *Pleurotus* spp. is very miscellaneous, reasons why many difficulties are encountered in correctly identifying species. However, some recent approaches have helped to unravel the biology, reproduction and diversification mechanisms of this gender. (Menolli, Suellen, Capelari, & Biologia, 2013). It is well known that the genus is

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promiscuous in terms of reproduction, which guarantees structural and physiological diversity. It is also known that the biological complexity of mushrooms of the genus *Pleurotus* spp. reflects in its chemical composition, mainly in primary metabolites, such as polysaccharide and proteins (Menolli et al., 2013; Silveira et al., 2015).

Given the scenario of mushroom related with the complexity of cell-wall, which the metabolites of interest are linked, the methodologies of polysaccharide extraction have been improved to facilitate the penetration of solvent into the concentration area of these metabolites (Chen et al., 2016). In this context, recent extraction methods such as subcritical water technology, pressurized hot water, and deep eutectic solvents can help the extraction of polysaccharides with higher yields, better purity, lower energy cost and less harmful to the environment, since these technologies are considered green (Jiao et al., 2017).

The polysaccharides of the genus *Pleurotus* spp. have several structural characteristics, however, can be observed mainly chemical structures of glucans with varying degrees of bonds and conformations. These complex polysaccharides, in terms of the organization of their monomers and bond types, are responsible for activating extensive pathway networks and biochemical defense mechanisms of the organismo (Singdevsachan et al., 2016). The polysaccharides of mushroom from the genus *Pleurotus* spp. may aid living organisms in various defensive routes against infections and mutations, thus acting as potent activators of the defense system, which ensures antitumor, antioxidant, immunostimulatory action among others (Zhu, Du, Bian, & Xu, 2015).

This genre has been investigated for over a century, the results of this immense effort in technology research and development are addressed in this bibliographic review. Therefore, this paper aimed to elucidate the main findings on the chemical composition of polysaccharides, structural, physical characteristics and bioactivities, emphasizing their mechanisms and action routes, new emerging techniques of extraction of polysaccharide and finally to address recent technologies under development with mushroom polysaccharides of the genus *Pleurotus* spp.

2. The genus *Pleurotus* spp. and its characteristics

The genus *Pleurotus* spp. comprises upper basidiomycete fungi forming oyster-bearing fruit bodies. The species of the genus are distributed all over the globe, colonizing several ecological niches, most preferably rotting tree trunks and branches, for this reason they are known as white rot fungi (Fernandes, Barros, Martins, Herbert, & Ferreira, 2015). It is estimated that there are more than 200 species of fungi of the genus *Pleurotus* spp., all of which are edible and appreciated for their taste, aroma, and texture, as well as the health-enhancing bioactive potentials (Valverde, Hernández-pérez, & Paredes-lópez, 2015).

The genus *Pleurotus* spp. has long been the subject of studies. Research groups around the world, most notably in Asia, have contributed substantially to groundbreaking research for the advancement of science, focusing primarily on areas such as carbohydrate chemistry from fruiting bodies and submerged crops, extraction of secondary metabolites (Alkaloids, flavonoids, betalains among others) and biomedical, pharmaceutical, biotechnological and food applications (Ren, Perera, & Hemar, 2012, 2015).

Another important aspect of current research involving fungi of the genus *Pleurotus* spp. is related to the delimitation of species within the genus. This issue has been discussed for decades by mycologists who point out the main causes of controversy related to species taxonomy and morphology of *Pleurotus* spp. Several reports of species identification of *Pleurotus* spp. have errors, which can be summarized and explained by the biological complexity of the genre. It is known that a single species can assume distinct morphological characteristics, depending on environmental changes, such as *Pleurotus* ostreatus, that can be found with the white or black pole, depending on the ambient light conditions (Menolli et al., 2013). Other aspects of morphology, physiology and even reproduction are factors that can lead to errors during identification. Higher fungi are known to be unisex, have many different ways of having sex, thus they are considered sexually promiscuous, but these peculiar characteristics make this kingdom one of the most successful on the evolutionary scale (Heitman, 2015; James, 2015).

However, even though morphological, reproductive and physiological characteristics lead to misidentification, modern techniques of biochemical and molecular approach have provided insight into the genus and its species, but these techniques are better when accompanied by results of morphology and reproduction (Menolli et al., 2013). Modern identification methodologies such as electrophoresis, amplified fragment size polymorphism (AFSP), ribosomal DNA sequencing, restriction fragment length polymorphism (RFLP) and reproduction testing, among others, are contributing to the confirmation of the taxonomic status of many species within the genus *Pleurotus* spp. (Maftoun et al., 2015). However, other, less invasive and highly specific techniques such as Fourier Transform Infrared Diffuse Reflectance (DRIFT) is a fast, easy, economical methodological option and can be used for classification of pure cultures of Fourier Transform species of *Pleurotus* spp. (Zervakis, Bekiaris, Tarantilis, & Pappas, 2012).

Among the many important aspects surrounding the genre *Pleurotus* spp. and which has a direct influence on its economic value and its qualification as a functional food, comes from the latest discoveries about its nutritional value. The genus is famous for having complete attributes for healthy eating. Among which we can highlight the nutritional value, taste, and aroma as well as bioactive functions. Volatile compounds such as terpenes and flavonoids, as well as biomolecules such as polysaccharides, proteins, and amino acids, contribute to a diversity of flavors, taste and texture characteristics (Gil-ramírez et al., 2017; Silveira et al., 2015; Smiderle et al., 2012). In this aspect the species *Pleurotus* ostreatus, perhaps the best known and most popular edible fungus of this genre has been used in many recipes and sophisticated dishes like soups and roasts. The *Pleurotus* eryngii (DC.) Quél., has also appreciated gastronomic attributes, besides being used in various dishes can be consumed in nature, preserving much of its sensory and functional characteristics (Sakamoto, 2018).

The nutritional quality of the species of *Pleurotus* spp. is already well understood, recent reports consider the genus to be low in fat, plus nutritional levels of carbohydrate, protein, amino acids and minerals well above many other sources. The amount of crude protein in mushrooms is below the meat, well above other vegetable sources and even meat products like milk. In addition to having all nine essential amino acids, which allows the replacement of meat by mushrooms in a balanced diet (Carrasco-gonzález, Serna-saldívar, & Gutiérrez-uribe, 2017). The presence of chitin in the cell wall of mushrooms brings extra benefits for food, as it is an important source of dietary fiber. Linked to these attributes the vitamin content rich in riboflavin, thiamine, niacin and ascorbic acid, as well as minerals such as phosphorus, iron, and magnesium increase the nutritional value of mushrooms of this genus *Pleurotus* spp. (Gil-ramírez et al., 2017).

The chemical analysis shows that among the species of *Pleurotus* spp. humidity levels are quite high, concentrated in a range of 85–90% of moisture, which depends on factors such as species, cultivation, harvesting and storage parameters (Atri, Sharma, Joshi, Gulati, & Gulati, 2013). Other parameters such as protein, carbohydrate, fiber and ash percentage are very diverse among mushrooms of this genus *Pleurotus* spp. For this reason, it is not worth making generalizations about composition. However, the following papers presented below provide insightful research into the chemical composition of several species of *Pleurotus* spp. (Atri et al., 2013; Reis, Barros, Martins, & Ferreira, 2012; Taylor, Khan, & Tania, 2012), and the revisions proposed by Maftoun et al. (2015) and Belletini et al. (2019) provides detailed information on the composition of amino acids, volatile compounds, fatty acids, vitamins, carbohydrates and their relationship to external factors. It's worth exploring these surveys and reviews

depending on the reader's need.

3. Techniques of extraction of new polysaccharides

The polysaccharides of mushroom can be obtained from fruiting bodies, mycelial biomass or directly from liquid culture broth, such as exopolysaccharides released into the extracellular medium (Ruthes, Smiderle, & Iacomini, 2015). There are currently several polysaccharide extraction techniques, such as hot water extraction (Maity et al., 2011a), microwave assisted extraction (Maity et al., 2011b), pulse extraction (Zhang, Zhang et al., 2018, 2018b), ultrasound assisted extraction (Kang et al., 2019), alkaline extraction (Liu et al., 2019a, 2019b), enzyme-assisted extraction (Khatun et al., 2015), among others, each with its own peculiarity, advantages and disadvantages.

All these techniques aim to extract complex biopolymers linked to the cell wall of mushrooms, considering their peculiarities related to the fungal cell wall, where polysaccharides and proteins are linked (Wu et al., 2019). Another important detail is related to the maintenance of polysaccharide properties, its molecular weight, structural conformation and type of peptide bonds, since these characteristics are mainly responsible for the bioactive properties of these biopolymers (Wang, Shi, Yin, & Nie, 2018; Yan, Meng, et al., 2019).

The cell-wall of fungal is a complex structure composed mainly of glucans (50–60%), glycoproteins (20–30%), lesser chitin (10–20%) and proteins. The supramolecular assembly of these molecules is not adequately elucidated, however it is known that the chitin microfibrils are arranged close to the plasma membrane. These chitin microfibrils may be covalently linked to the β- (1 → 3) glucans, spread across the cell wall, where they may make bonds, or even weak interactions with functional groups of mannanoproteins, which are attached to these structures. In addition to the linear fibril and glucan network, branched β- (1 → 6) glucan networks act as bridges helping to keep the cell-wall structure compact (Kang, Kirui et al., 2018).

Thus, extraction techniques must be energy efficient to break through and penetrate the dense canals of glucans and glycoproteins, solubilizing them and extracting these metabolites (Zhang, Li et al., 2016, 2016b; Zhang, Yang et al., 2016). Parameters such as solvent type, extraction time, temperature, pressure and pH should be taken into account for polysaccharide extraction, as well as matrix characteristics such as density, porosity and humidity. However, as a basic rule, the principle of each extraction depends on the equipment, however, the extraction fundamentals always occur by the following steps, not necessarily in this order: first the fluid rises the determined working conditions moistens the matrix, penetrating through the pores and molecular channels to reach the cell wall, where various mass transport phenomena (biochemical and physical) help penetrate the cell wall. Several phenomena occur until the cell wall is ruptured, and its metabolites are transfered out of the cellular environment, from where they are carried by the extracting fluid. Additional steps of separation and purification of these metabolites are important for obtaining pure fractions (Chen et al., 2016; Cocero et al., 2018).

The most widely used technique for polysaccharide extraction is aqueous extraction. The technique is based on the polarity principle of polysaccharide molecules that are compatible with the polarity of water, so they are solubilized during the extraction process (Ruthes et al., 2015). The mushroom fruit bodies can first be extracted with some organic solvent, usually ethanol or acetone to remove nonpolar compounds, the organic solvent facilitates the complete separation of polysaccharides from other compounds such as lipids, phenols, and terpenes, although this step is not required (Maity et al., 2011b). Polysaccharides extraction is traditionally performed with hot water (90–100 °C), this procedure can be repeated until the exhaustion for a period ranging from 2 to 5 h. Then, this material is centrifuged and separated from the solid fraction of the mushroom fruit bodies to obtain the concentrated aqueous extract of polysaccharides (Ruthes et al., 2015).

Table 1
Main extraction methods of *Pleurotus* spp. polysaccharides reported in the literature.

Extraction Method	Mushroom	Time of Extraction (min)	Temperature/ pressure/ Power	Yield %	Reference
Hot Water	*Pleurotus* ostreatus	240	100 °C – –	0.88	Yan et al., 2019a
	Pleurotus eryngii	120	100 °C – –	29.2	Xu et al., 2016a
	Pleurotus eryngii	180	95 °C – –	7.31	Li & Shah, 2016
Alkaline	*Pleurotus* eryngii	300	90 °C – –	12.18	He et al., 2016
	Pleurotus tuber-regium	120	– – –	0.44	Wu et al., 2013
	Pleurotus florida	30	100 – –	0.08	Maity et al., 2011b
Ultrafiltration	*Pleurotus* eryngii	140	70 °C – –	10.90	Ma et al., 2016
Ultrasonic	*Pleurotus* eryngii	170	80 °C – –	7.5	Liu et al., 2010
Microwave Assisted	*Pleurotus* ostreatus	30	180 °C 1.5 Mpa 850 W	32.4	Smiderle et al., 2017
Enzyme Assisted	*Pleurotus* djamor	44,77	35.36 °C – –	3.61	Jiao et al., 2017

Other techniques such as microwave assisted extraction, pulse extraction technology and ultrasound assisted extraction have been used. However, these methods have disadvantages such as long extraction times, low efficiency and risk of degradation of biopolymers (Zhang, Li et al., 2016, 2016b; Zhang, Yang et al., 2016). In addition to these, more modern techniques such as subcritical water extraction (SWE) and the use of deep eutectic solvents are being used for polysaccharide extraction, these two techniques will be more detailed below (Zdanowicz, Wilpiszewska, & Spychaj, 2018). Table 1, below summarizes the main polysaccharide extraction techniques.

3.1. Subcritical and pressurized hot water extraction technology

Water is considered a “green” solvent capable of adapting its properties through thermodynamic process change, thus being able to be used in polysaccharide extraction under conditions above normal temperatures and pressures (273.16 K and 101,325 Pa, respectively) (Dey et al., 2012). Recent data from the specialized literature, such as articles on lignocellulosic biomass fractionation (Cocero et al., 2018; Takada, Minami, & Saka, 2018), supercritical biomass gasification and generation of value added products with subcritical water technology (Alonso, 2018; Knez, Hrnčič, Čolnik, & Škerget, 2018; Rodriguez Correa & Kruse, 2018; Sunphorka, Chavasiri, Oshima, & Ngamprasertsith, 2012), hydrothermal liquefaction (2019, Cantero-Tubilla et al., 2018; Chan et al., 2018) and polymer extraction and carbohydrate depolymerization (Dulay et al., 2017; Limarta et al., 2018; Pérez & Tuck, 2018; Pérez, Tuck, & Poliakoff, 2018; Taofiq et al., 2015), demonstrate that water under specific temperature and pressure conditions undergoes drastic changes in its physical, chemical and molecular characteristics, which contributes to the specificity of extraction.

Water is recognized as a universal solvent, however, because of its properties as polarity and high dielectric constant, it cannot be used for extraction of nonpolar metabolites, and even some polar molecules

have enough strength not to be extracted by water under conditions of normal pressure and temperature. In this sense, supercritical and subcritical fluid technology can be a useful technological tool for the extraction of primary mushroom metabolites. A supercritical fluid is defined as any substance that is subjected to a temperature and pressure above its critical values. In the case of water, its critical temperature is 374 °C and the critical pressure is 22.1 Mpa (Plaza & Turner, 2017).

When the water temperature is above its boiling point (between 100 and 374 °C), plus the pressure is maintained under controlled conditions to keep the water in a liquid state, its physical and chemical properties change dramatically. Under these conditions (pressurized hot water and subcritical water), both defined from the proximity of the critical point parameters (temperature and pressure), the water acts as an organic solvent and can dissolve a diversity of molecules (Plaza & Turner, 2017).

Extraction process parameters such as temperature, pressure, solvent flow, bed packaging, bed type, particle size, solvent type and its physicochemical characteristics, pH and extraction time directly influence the yield and efficiency of the extraction process. Extraction process temperature directly influences yield through physicochemical solvent changes, including dielectric constant and solubility. Increased water temperature causes density reduction, providing low viscosity, increased vapor pressure and higher mass transfer rate, which contributes to high overall yields compared to conventional polysaccharide extraction methods (Zhang et al., 2019).

The extraction pressure has a direct influence on the rate of mass and energy transfer during extraction, besides contributing to the acidification of the extraction medium. The increase in [H +] ions acidifies the medium, favoring bond breaking mechanisms and increasing polysaccharide extraction rates. Viscosity and diffusivity directly affect the solubility of the extraction medium, these parameters are affected by the pressure and temperature of the process. Due to these thermodynamic results, it is consistent that these parameters are properly controlled throughout the extraction process. (Askin, Sasaki, & Goto, 2010).

The pressurized hot water and subcritical water have been used in several extraction works of polysaccharides and sugars from various matrices as plants (Chao, Ri-Fu, & Tai-Qiu, 2013; Ravber, Knez, & Škerget, 2015; Shimanouchi, Ueno, Yang, & Kimura, 2014; Zhang et al., 2019), animals (Getachew, Lee, Cho, Chae, & Chun, 2019) and mushroons (Askin et al., 2010; Benito-Román, Alonso, Cocero, & Goto, 2016; Luo et al., 2017; Smiderle et al., 2017; Zhang, Zhang et al., 2018, 2018b). Others also evaluated the addition of supercritical carbon dioxide to the process. According Benito-Román et al. (2016), the addition of supercritical carbon dioxide to the pressurized hot water polysaccharide extraction process results in improvements in the extraction yield by acidification of the aqueous medium. Overall this technology is quite versatile as it does not require many sample treatments before and after extraction. One of the most promising advantages of pressurized hot water and subcritical water extraction techniques is that the raw material does not need to be dried or lyophilized to extract, then self-moisture extraction can be done (Machmudah & Goto, 2013; Machmudah, Kanda, Sasaki, & Goto, 2015).

Polysaccharides were extracted from the mushroom *Pleurotus* ostreatus by extraction with pressurized hot water. The ground mushroom was mixed with 1: 4 sea sand (1 g sample: 4 g sand) and extracted with pressurized water at 106.8 bar and temperatures between 100 and 200 °C. The sea sand is used to increase porosity of the extraction bed and to decrease preferred solvent paths during polysaccharides extraction. Extraction water temperature is a very important parameter, as the solubility of the polysaccharide depends on its molecular structure and temperature. In fact, the highest yields were observed at the highest temperatures used during the process. The temperature of 200 °C obtained the highest yield, reaching 78.6%, demonstrating that the extraction method with pressurized water is efficient and can be used for polysaccharide extraction. However, degradation products were observed at this temperature, which may be a limiting factor for yield (Li & Shah, 2016).

Smiderle et al. (2017) evaluated the extraction of β-glucans from *Pleurotus* ostreatus by subcritical water. Extractions were performed in an accelerated solvent extractor. An 11 mL extraction cell was loaded with a cellulose filter and filled with crushed mushroom and 1: 8 washed sea sand (mushroom: sand), the extraction procedure was performed with pressures ranging from 10.2 to 11.7 Mpa according to the experimental design of surface response methodology (RSM). The extraction yield was 40%, exceeding the value predicted by the experimental design which was 32%. The optimized process conditions were 180 °C for 26 min. The yield was associated with the extraction method, since water under subcritical conditions is capable of extracting hydrophobic compounds, as well as insoluble polysaccharides, such as linear β-glucans and chitins, in which case the temperature had a strong positive influence, as well as the time of the extraction affected the polysaccharide yield. Pressure-associated temperature affects mass transfer rate, which favors extraction, solubility and diffusion coefficient

3.2. Deep eutectic solvent extraction technology

In current research reports the green chemical concept has been highly valued and employed to describe extraction processes with little or no adverse effects on the environment. In this context, deep eutectic solvents (DESs) are one of the main solvents with similar physicochemical properties to ionic liquids, with some advantages, such as ease of synthesis and lower cost (Zdanowicz et al., 2018; Zhang et al., 2017). When compared to subcritical and supercritical extraction fluid technology, deep eutectic solvents have some advantages, such as low pressures and extraction temperatures. In addition, they have higher carbohydrate polymer recovery yields, mainly due to the high solubility of polysaccharides in eutectic solventes, such as CC / citric acid monohydrate, CC / oxalic acid dihydrate and CC / ethylene glycol. (Zdanowicz et al., 2018). Deep eutectic solvents (DESs) are arrangements made from a eutectic mixture of Brønsted-Lewis bases and acids, hydrogen bond acceptors and hydrogen bond donor that has various types of cationic or anionic groups (Cui et al., 2017). The DES such as CC / acetic acid, CC / formic acid, alanine / lactic acid can be prepared from natural compounds or purchased by simple heating and mixing techniques, such as CC / urea, dimethyl urea / $ZnCl_2$, and chlorocholine chloride / urea. Quaternary ammonium salts (hydrogen bond acceptor), amines, amides, carboxylic acids and polyols (hydrogen donor) are used as cationic and anionic groups (Shishov, Bulatov, Locatelli, Carradori, & Andruch, 2017). Due to properties such as low vapor pressure, chemical and thermal stability, non-flammable, high dissolving capacity, low melting point, biodegradability, non-toxic, polarity, recyclability and low cost, deep eutectic solvents are a innovative technology for polysaccharide extraction (Cui et al., 2017; Shishov et al., 2017; Zdanowicz et al., 2018).

The use of deep eutectic solvents in polysaccharide extraction has been clarified in several recent publications (Liu et al., 2010; Zhang et al., 2017; Zulkefli, Abdulmalek, & Abdul Rahman, 2017) and the Review proposed by Zdanowicz et al. (2018). However, this technology has been little explored in the extraction of mushroom polysaccharides, especially those of the genus *Pleurotus* spp., which sets the precedent for further investigations and the possibility of a new field of research and applications.

4. Structural characteristics, chemical and physical properties

Several complex structures of mushroom polysaccharides of the genus *Pleurotus* spp. have already been elucidated, most of these structures are β-glucans. Fungal glucans are variable polymeric structures of D-glycopyranose (D-Glc p), important constituents of fungal cell biology, and are the dominant chemical structures of the mycelium

cell wall and other micro and macromicete cell structures (Cortés, Curto, Carvalho, Pérez, & Ribas, 2019; Fesel & Zuccaro, 2016; Geoghegan, Steinberg, & Gurr, 2017; Hopke, Brown, Hall, & Wheeler, 2018). In addition to fruit body polysaccharides, other polysaccharides (duly called exopolysaccharides) produced in submerged cultivation under controlled conditions are characterized as chemical structures of β-glucans (Wang, Salem, & Sani, 2019; Wang, Li, Chen, & Han, 2012; Yildiz & Karatas, 2018).

Although the monosaccharide composition of β-glucans is very simple, by definition they consist basically of glucose monomers, a large number of chemical structures can be found, as these monomers may have different anomeric configurations of Glcp units, degree of branching (with several side chains, one or more monosaccharides linked at different positions), chain conformation, molecular weight, position and glycosidic bonds along the chain (Imre, García, Puglia, & Vilaplana, 2019; Ruthes et al., 2015; Synytsya & Novák, 2013; Zhu et al., 2015).

For ease of understanding, fungal polysaccharides are divided into three main groups, this classification is based on the anomeric structure of the D-glycopyranose (D-Glcp) monomers, so α-D-glucans are distinguished; ρ-D-glucans and the mixed chains called β-D-glucans (Ruthes et al., 2015). Another form of discrimination of glucans is based on the position of the glycosidic bond and the molecular weight, ie main chain glucans, with the linked monomers (1 → 6), (1 → 3) and the position of the branches as β- bound glucans (1 → 6), (1 → 3) and (1 → 4), medium and high molecular weight, and classification by bioactivity as anti-tumor, immunostimulatory, anti-inflammatory, antiviral β-glucans among others (De Arcangelis et al., 2019; Maheshwari, Sowrirajan, & Joseph, 2019; Zhu & Wu, 2019; Zou, Duan, & Xu, 2019).

Wide range of β-glucan-like polysaccharides, such as α-D-glucan (1 → 3) - bound (Bhanja et al., 2014; Nie, Zhang, Li, & Xie, 2013), β-D-glucan (1 → 3) - bound (Rosado, Cosentino, Alquini, Carbonero, & Iacomini, 2004) and β-D-glucan (1 → 6) - bound (Smiderle et al., 2013), have already been elucidated in various types of mushrooms as, Agaricus brasiliensis, Agaricus bisporus, Coprinus comatus and Laetiporus sulphureus. In addition to the diversity of natural polysaccharide structures, several studies have made chemical modifications to the β-glucan structures, with the addition of microelements and other functional groups, obtaining more water-soluble functionalized derivatives with greater bioactivity (Li, Chen, Chen et al., 2018; Malinowska et al., 2018; Wang, Zhang, Shao, Wu, & Li, 2019).

The chemical structures of β-glucans, purification techniques and structural analysis of various microorganisms have been adequately addressed in recent review papers (Deniaud-Bouët, Hardouin, Potin, Kloareg, & Hervé, 2017; Ferreira, Passos, Madureira, Vilanova, & Coimbra, 2015; Ruthes et al., 2015; Sovrani, de Jesus, Simas-Tosin, Smiderle, & Iacomini, 2017; Synytsya & Novák, 2013; Zhou, Li et al., 2019; Zhou, Cui, & Qu, 2019), where the structural and conformational details of these complex molecules have been properly reported. However, the multi-structure approach does not emphasize specific groups of any kind. Thus the approach of β-glucan molecules of the genus *Pleurotus* spp. must be properly addressed and detailed. The following will be discussed examples of several β-glucans of the genus *Pleurotus* spp., emphasizing its structural, informational and physicochemical properties.

The mushroons *Pleurotus* eryngii and *Pleurotus* ostreatoroseus, were used for polysaccharide extraction with hot water. The extracted polysaccharides were freeze-thaw fractionated and their chemical structure was elucidated by a set of spectroscopic techniques, Smith degradation, and methylation analysis. The results identified a branched β-glucans (Fig. 1), with a major chain of (1 → 3) - Glc p residues, substituted at the O-6 position by (1 → 6) - Glc p side chains, unitary, on average every three skeletal residue (Carbonero et al., 2006). This type of polysaccharides with substitution degree every three residues has been elucidated in several basidiomycetes. Similar structure was found for *Pleurotus* ostreatus (Yoshioka, Tabeta, Saitô, Uehara, & Fukuoka, 1985), *Pleurotus* pulmonarius (Smiderle et al., 2008, 2012), *Pleurotus* citrinopileatus (Liu et al., 2012), Auricularia auricula-judae (Miyaji, 1981) and Boletus erythropus (Chauveau, Talaga, Wieruszeski, Strecker, & Chavant, 1996).

The hot aqueous extraction of the basidiocarps from the mushroom *Pleurotus* sajor-caju provided a water-soluble, gelatin-like glucan that was chemically characterized as a highly branched glucan. The structure was elucidated (Fig. 2) as (1 → 3) - β - Glc p in the main chain, replaced at the O-6 position by unit of (1 → 3), (1 → 6) - β - Glc p in the lateral chains, on average two out of three residues in the spine, with a molar mass of 9.75×10^5 g / mol^{-1} (Carbonero et al., 2012).

Other chemical structures have already been elucidated from *Pleurotus* sajor-caju, such as heteropolysaccharides (Fig. 3), D-glucose, D-galactose and D-mannose, in a 1: 1: 1 M ratio (Pramanik, Mondal, Chakraborty, Rout, & Islam, 2005; Roy, Maiti, Mondal, Das, & Islam, 2008) and D-glucans with α and β-Glc p-type units, as well as Glc p-linked units (1 → 2) (Pramanik, Chakraborty, Mondal, & Islam, 2007).

Other polysaccharides, such as methylates are being reported for mushrooms of the genus *Pleurotus* spp., like the ones highlighted for the mushroom *Pleurotus* citrinopileatus. A partially 3- O-methylated linear α-galactopyranine (Fig. 4) consisting of α-galactose (1 → 6) bound, 6-O-methyl galactose bound to (1 → 6) and glucose bound to (1 → 6) 4) at a molar ratio of 3.0: 1.0: 0.6, with a molecular weight of 27.4 kDa, was elucidated (He et al., 2016). Two other partially methylated galactans were isolated from the fruit bodies of this fungus by extraction with hot water, followed by freeze-thaw fractionation and precipitation. A set of one- and two-dimensional spectroscopic techniques, methylation analysis and monosaccharide composition characterized the polysaccharides as linear α-galactopyranes, partially 3-O-methylated (1 → 6), containing only Gal and 3-O-Me-Gal, molar proportions 2: 1 and 1: 1, with molar masses of 37.6×10^3g / mol and 28.5×10^3g / mol, respectively (Brito et al., 2018).

A branched glucan soluble in an aqueous sodium chloride solution was extracted from the fruiting bodies of *Pleurotus* florida. The structure was established to be a (1 → 3) - α-D-Glc p-linked glucan, (1 → 3) - β-D-Glc p-linked, with branching (1 → 6) - α- Gl-Glc p (Rout, Mondal, Chakraborty, Pramanik, & Islam, 2005). Glucans were isolated from the fruiting bodies of *Pleurotus* ostreatus and *Pleurotus* eryngii by subsequent boiling with hot water and alkaline extraction. In the water-soluble (L1) and insoluble (S) fractions, predominated (1 → 3) and (1 → 6) - branched β-D-glucan, while in the alkaline (L2) soluble fraction, predominated (1 → 3) α- Linear D-glucan (Synytsya et al., 2009).

5. Biological activities and their mechanisms

The fungal polysaccharides have revealed a whole new range of multivariate bioactivity biomolecules such as antiviral, antifungal, anticancer, antidiabetic, anti-inflammatory, and many other pharmacological activities in the treatment of various human diseases (Datta et al., 2019; Du, Zhu, & Xu, 2018; Rathore, Prasad, & Sharma, 2017, 2019; Reis et al., 2017; Wu et al., 2019). Table 2 presents several macrofungus of the genus *Pleurotus* spp. with their respective bioactivities.

5.1. Antitumor activity

The cancer is one of the most serious diseases today, considered the leading cause of death worldwide, accounting for over 8.2 million deaths in recent years (Li, Shen, Nie, Duan, & Chen, 2019; Saner et al., 2019). Malignant tumors are the most severe form of the disease, as they initiate a series of cascading cell uncontrols, increasing levels of free radicals that accelerate cell uncontrollability, with catastrophic results such as tissue destruction and disruption of cell membranes, causing the cell north (Aspesi & Ellis, 2019; Karki & Kanneganti, 2019; Salmon, Remark, Gnjatic, & Merad, 2019). Understanding tumor mechanisms and their effects on other cells, as well as their mutagenesis

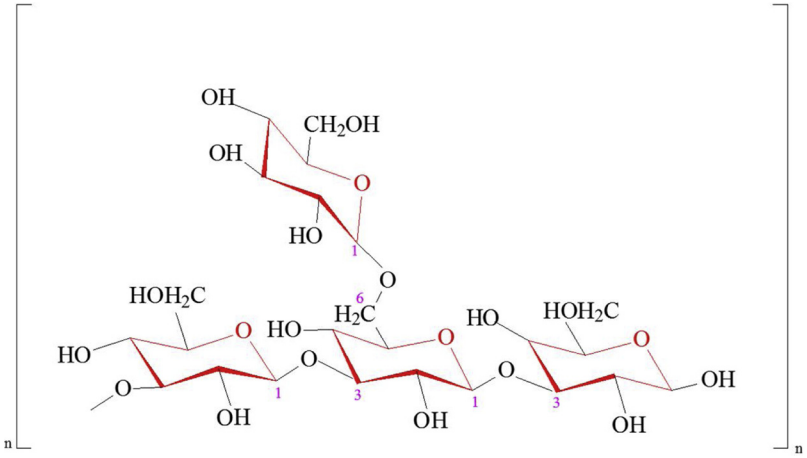

Fig. 1. Chemical structure of a branched main chain (1 → 3) - Glc p and (1 → 6) - Glc p side chains, branched β-glucans.

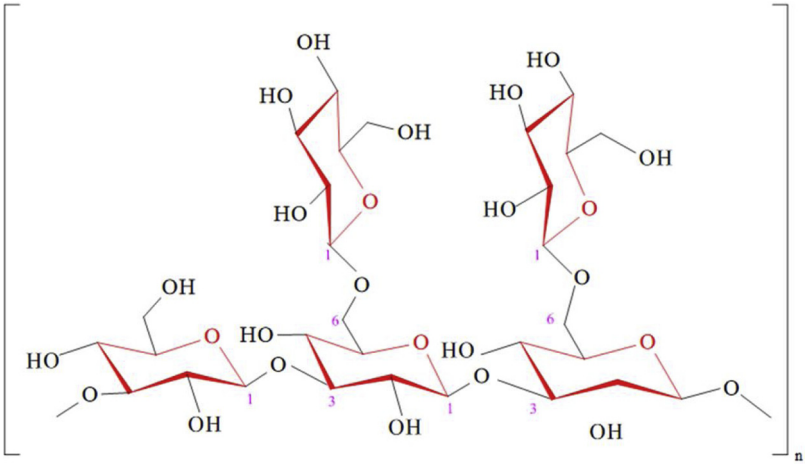

Fig. 2. Branched structure with a (1→3) -linked β-Glcp main-chain, substituted at O-6 by single-unit β-Glcp side-chains, on the average of two to every third residues.

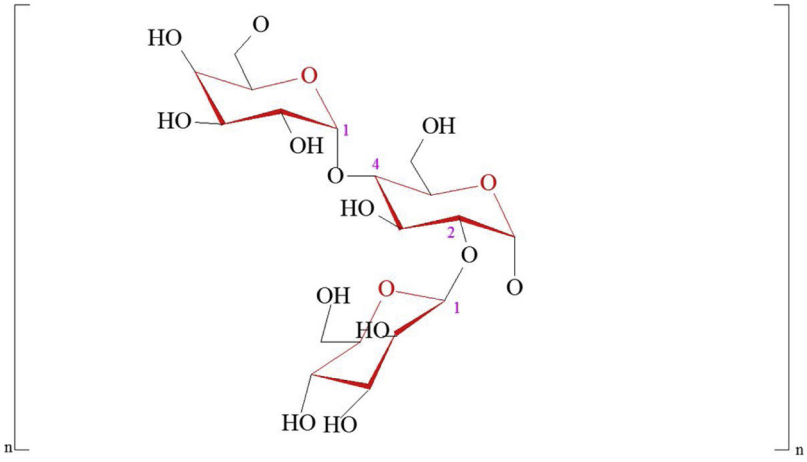

Fig. 3. Chemical structure of a branched (1 → 6) - α - Gal p- (1 → 4) - α - Glc p and (1 → 2) β - Man p side chain branched heteropolysaccharide.

processes, is the key to the development of efficient drugs and chemotherapy (Aspesi & Ellis, 2019).

The tumor formation and its progression to malignancy depends on inflammatory stimuli in a set of cells that acquire mutations in oncogenesis or tumor suppressor genes (Altorki et al., 2019; Huynh, Gough, & Ernst, 2019). Although current research focuses on changes related to oncogenesis, it is known that several natural (nonmalignant) cells without inflammatory stimuli within the cellular microenvironment may evolve along with cancerous tumors, acquiring the malignant phenotype (Altorki et al., 2019; Lens, 2019). This evolution from healthy to tumor cells is essential for cancer progression. In fact, both the cellular (systemic) and local environment are influencers of cancer, and play a crucial role in tumor initiation through the generation of systemic inflammatory responses to various punctual stimuli (Mossmann, Park, & Hall, 2018; Zinzalla, Stracka, Oppliger, & Hall, 2011).

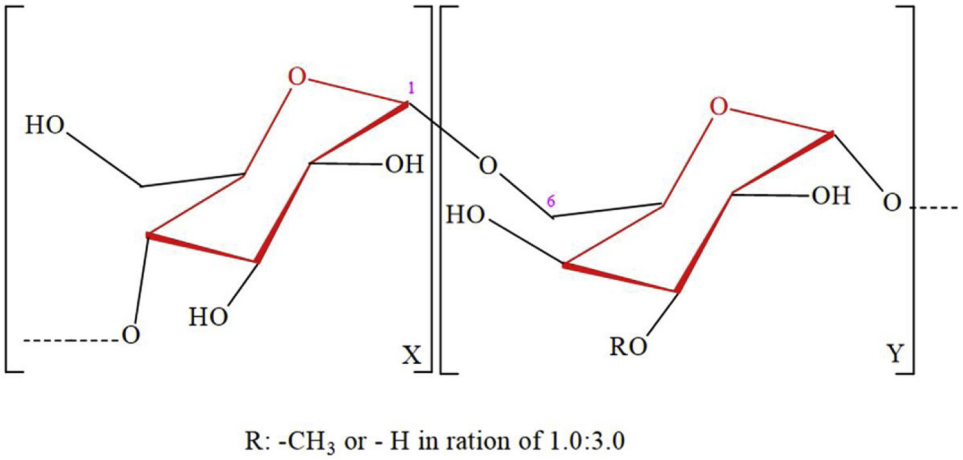

Fig. 4. Chemical structure of a linear α-glycopyranose: (1 → 6) - α - Gal p- (1 → 6) - α - Gal p- (1 → 4) -α - Glc p.

There are several types of stimuli that can lead to a picture of cellular inflation. For example, persistent inflammations such as those caused by microorganisms are considered cancer initiators (Mertens, Johansson, Fioretos, & Mitelman, 2015; Sondka et al., 2018). Latent inflammation from viral infections such as human immunodeficiency virus (HIV) and hepatitis B virus, which causes widespread liver infections, as well as bacterial infections such as bacteria Helicobacter pylori in the stomach, or even continued exposure to external factors such as radiation and toxic chemicals, are considered factors that are associated with cancer initiation (Dang et al., 2009; Pang et al., 2009; Xu et al., 2013).

Furthermore, inflammatory conditions are known to have substantial macrophage action through various cytokines capable of causing tumor cell death (apoptosis) and to have a wide range of proinflammatory actions. These beneficial effects will be more clearly explained below. However, at present macrophages will be discussed as correlating between macrophage-mediated inflammation and cancer induction. Deng et al. (2009), will confirm the macrophage-mediated carcinogenic activity with the chronic inflammatory colonic response following genetic ablation of the anti-inflammatory transcription factor Stat3, which was sufficient to induce the formation of invasive adenocarcinoma. Zhou et al. (2014) and West, Mccuaig, Franchini, and Powrie (2015), corroborates the correlation between macrophage-mediated inflammation and cancer induction through the loss of anti-inflammatory cytokines (IL-10), which is produced by the anti-inflammatory transcription factor Stat3, as a result of increased

Table 2
Main bioactivity of polysaccharides of the genus *Pleurotus* spp.

Mushroom	Bioactivity	References
Pleurotus ostreatus	Antihyperlipidemia	Dong et al., 2019; Zhang et al., 2017
	Antioxidant	Khan et al., 2017; Ma, Zhao, Yu, Ji, & Liu, 2018; Yan et al., 2019a, 2019b, 2019c
	Antitumor	Facchini et al., 2014; Kong et al., 2014; Zhang et al., 2012
	Anti-inflammatory	Taofiq et al., 2015
	Antidiabetic	Zhang, Li et al., 2016
	Immunomodulator	Vetvicka et al., 2019
	Cognitive impairment	Zhang, Li et al., 2016, 2016b; Zhang, Yang et al., 2016
	Gastroprotective	Yang et al., 2012
Pleurotus eryngii	Antioxidant	Ren et al., 2017; Yan et al., 2019a, 2019b, 2019c; Zhang, Li et al., 2016, 2018a; Zhang, Wen et al., 2018
	Immunomodulator	Sun & Li, 2017; Vetvicka et al., 2019; Xu et al., 2016
	Antihyperglycemia	Chen et al., 2012; Ren et al., 2017; Zhang, Zhang et al., 2018, 2018b
	Renoprotector	Zhang, Zhang et al., 2018, 2018b
	Hepatoprotectants	Chen et al., 2012; Zhang, Li et al., 2016
	Antitumor	Ma et al., 2016, 2014
	Biological control	Sufiate et al., 2017
Pleurotus sajor-caju	Antioxidant	Boonsong & Klaypradit, 2016; Finimundy et al., 2013
	Antitumor	Finimundy et al., 2018; Finimundy et al., 2013; Telles et al., 2011
	Anti-inflammatory	Silveira et al., 2015; Silveira et al., 2014
	Immunological	Carbonero et al., 2012
	Macrophage Activator	Yan et al., 2019a, 2019b, 2019c
	Antidiabetic	Kanagasabapathy, Chua, Malek, Vikineswary, & Kuppusamy, 2014
	Anticoagulant	Telles et al., 2011
	Antiproliferative	
Pleurotus abalonus	Antioxidant	Ren et al., 2015; Wang et al., 2012; Zhang et al., 2013
	Antitumor	Ren et al., 2015
Pleurotus djamor	Antioxidant	Dulay et al., 2017; Jiao et al., 2017
	Antibacteriana	Dulay et al., 2017
Pleurotus florida	Antioxidant	Khatun et al., 2015; Maity et al., 2011a, 2011b
	Immunological	Dey et al., 2013, 2012
	Biological activity	Panda et al., 2017
	Immunostimulant	Maity et al., 2011
Pleurotus Pulmonarius	Antioxidant	Khatun et al., 2015
Pleurotus citrinipileatus	Antioxidant	Khatun et al., 2015; Liu et al., 2019a, 2019b
	Hepatoprotective	Liu et al., 2019a, 2019b

carcinogen-induced tumorigenesis.

Inflammation is the initial cause of tumor formation, creating an environment conducive to mutagenesis, either directly through the generation of free radicals associated with multiple stimuli or even indirectly by the action of genotoxic bacteria on the epithelial cells, which lead to changes in the microbiome. leading to changes in the functions of cell barrier systems (Hasselluhn, Schmidt, Ellenrieder, Johnsen, & Hessmann, 2019; Tukenmez, Aktas, Aslim, & Yavuz, 2019). In this context, macrophages play a crucial role in the discussion of cancer initiation, as these cells produce growth factors (cytokines) that stimulate other cells to develop. The growth of cells near cancer cells enables these healthy cells to spontaneously acquire cancer-associated mutations (Coppé et al., 2019; Miedel et al., 2019). The mutations are perhaps one of the biggest challenges facing cancer today, as mutated cells can cause the recruitment of inflammatory cells, which contributes to a vicious cycle that only tends to drive cancer progression (Chou et al., 2019; Chowdhry et al., 2019; Sellers et al., 2019).

In current cancer research reports (Errico et al., 2019; Linde et al., 2018; Liu et al., 2019a, 2019b; Lu, Lai, Huang, Lee, & Chuang, 2018), There is sufficient data to indicate that macrophages play a relevant role in the onset of cancer, mainly because of their central status in inflammation-mediating biochemistry. However, what does not seem to be clear is whether macrophages in some inflammatory situations act in favor of the organism by killing the mutant cells before they become tumorigenic and thus having antitumor activity (Lee, Lin, & Lin, 2018). In this context, fungal polysaccharides, in particular mushroom α and β-glucans of the genus *Pleurotus* spp., It has shown that they may act as modifiers of the host's biological activity by activating the immune system, or even by other mechanisms that will be more clearly discussed below. Thus, macrophages activated by biochemical stimuli generated by polysaccharides can act in favor of the organism by killing the mutant cells before they become tumorigenic, or even during inflammatory processes already established as cancer cells, thus having antitumor activity (Khan, Date, Chawda, & Patel, 2019; Wu et al., 2019; Zong, Cao, & Wang, 2012).

It is well known that polysaccharide bioactivity against tumor cells is affected by polysaccharide structure and conformation, molecule size, conformation (double helix, triple helix and random coil), branching degree and water solubility, which affect its bioactivity (Ji et al., 2017). Generally, but not a general rule, it is known that the higher the molecular weight and the greater the solubility in water, the greater the antitumor bioactivity, so branched polysaccharides are more water soluble and therefore easier to transport to the primary target (Ren et al., 2012). Table 3 presents some polysaccharides of fungi of the genus *Pleurotus* spp. with bioactivity for various types of cancerous tumors.

The mechanisms of action of polysaccharides from mushrooms of the genus *Pleurotus* spp., were evaluated through various studies using cell lines in vitro and others tracked through studies in vivo, using appropriate animal models. The main mechanisms already reported indicate that polysaccharides act by inducing tumor cell apoptosis, cell cycle arrest, nitric oxide pathway and mitochondrial membrane depolarization, as well as by the immunomodulation mechanism (Khan et al., 2019). In addition to these mechanisms, polysaccharides can act through multiple pathways as well as have antioxidant activities that help prevent cancer (Khan et al., 2019; Wu et al., 2019).

The antitumor activity can occur by inducing tumor cell apoptosis or inhibiting the expression of cellular oncogenesis as follows: polysaccharides activate defense cells (macrophages, T lymphocytes, B lymphocytes, cytotoxic T lymphocytes (CTL) and natural killer), to express biochemical compounds such as cytosines (TNF-α, IFN-c, and IL-1β) and chemokines (Cai et al., 2012). These substances have innate antiproliferative activity, causing apoptosis and differentiation in tumor cells, as well as secreting reactive nitrogen, oxygen intermediates and interleukins (Kulkoyluoglu & Madak-Erdogan, 2016; Elmore, 2007; Wu, Sun, & Wang, 2017). Fig. 5, shows the mechanism of action of polysaccharides via tumor cell apoptosis induction.

In multicellular organisms, cell proliferation, differentiation, and death are regulated by specific gene groups that control each step in addition to its metabolic intermediates, which maintain tissue homeostasis (Baxter et al., 2019; Ellis et al., 2019; Jiao et al., 2017; Quintela-lópez et al., 2019). When these systems are operating normally all cell cycle steps are activated smoothly. However, when there is some disturbance of the cellular system, as discussed above, through stimuli that lead to the initiation of an environment conducive to mutagenesis, cell cycle manipulation may prevent or induce an apoptotic response (Hardy et al., 2019; Kim, Ahn, Jung, Lee, & Lee, 2019; Shirotani et al., 2019). Polysaccharide studies have shown that these metabolites can disable a number of tumor suppressor genes, such as the tumor protein gene (p53), the dominant oncogen, c-Myc, the retinoblastoma protein (RB) gene, and cyclin-dependent kinases. (Cdks), bringing cell cycle arrest in phase G2/M, phase S or phase G0/G1 (Koedoot et al., 2019; Tang et al., 2019; Tomás-loba et al., 2014; Walter et al., 2019).

The mushroom polysaccharides of the genus *Pleurotus* spp., may also mediate apoptosis responses in cancer cells through caspase protease family enzyme complexes, through the caspase-3 and caspase-9 activation cycle (Cao, Liu, Yang, Hou, & Li, 2015; Cui et al., 2014; Ren, Wang, Guo, Yuan, & Yang, 2016). In addition to all the mechanisms already reported, activation of the nitric oxide pathway in defense cells may aid in the reduction, or even cell death, of cancerous tumors (Wang, Cao, Zhu, Gu, & Zhu, 2016). As is well known, nitric oxide has the potential to kill cells as it is a very toxic and reactive radical. Thus, this property has been used and explored as a route of interest in cancer therapy (Yan et al., 2019b). Kong et al. (2014) observed from in vitro studies (Sarcoma-180 cells) anticancer effects of the polysaccharide fractions of the mushroom *Pleurotus* ostreatus. The results showed that the polysaccharide fraction (WPOP-N1) enhanced macrophage activation. Activated macrophages have a central role in regulating the immune response against tumors by producing various mediators such as TNF-α and NO. Analysis of the WPOP-N1 assays significantly improved macrophage phagocytosis and the release of effector molecules produced by macrophages, such as TNF-α and NO. Suggested results for macrophage activation are mainly mediated by recognition of polysaccharides by certain receptors, such as Toll-like receptor 4 (TLR4), receptor complement 3 (CR3), receptor sequester, dectin-1, and mannose receptor.

Yan et al. (2019a) extracted and purified a 3-O-methylated heterogalactan (WPEP-Nb, Mw 21.4 kDa) from the fruiting bodies of *Pleurotus* eryngii. In vitro studies have shown that WPEP-Nb increased macrophage phagocytosis and the release of effector molecules, such as NO, TNF-α, IL-6. Furthermore, activation of RAW264.7 cells can be mediated by the MAPK and NF-κB signaling pathways and the Toll-like 2 receptor (TLR2). In in vivo models cell death is induced after increased NO levels from induced nitric oxide synthesis (iNOS), which acts as an important messenger of physiological functions (Zhang, Zhang et al., 2018, 2018b). Thus, the increased production of nitric oxide (NO) in the cellular microenvironment causes tumor cell death through the caspase protease family enzyme complex pathway, this mechanism is summarized in Fig. 6.

The nitric oxide pathway is quite complex and involves a set of protein-dependent biochemical mechanisms and specific enzymes such as caspase proteases. NO can act directly on cancer cells by specific biochemical pathways, such as by activating priming enzymes that can inhibit the action of essential enzymes for cancer cell metabolism (Ma et al., 2014; Yan et al., 2019b). In addition, it can act to deplete deposits of antioxidant compounds, inducing the biochemical pathway of lipid peroxidation as well as causing irreparable DNA damage, leading to unscheduled cell death (Sun & Li, 2017; Thangam et al., 2014; Wu et al., 2012). Caspases play a fundamental role in the polysaccharide-mediated cell apoptosis modulation cycle. Two major components can be observed, caspase-8 acts as an initiator by remediating the Faz protein-induced biochemical pathway of cell death, whereas caspase-9

Table 3
Polysaccharides of *Pleurotus* spp. mushrooms with antitumor activity.

Mushroom	Fraction/ Extraction	Name Fraction	Dosage (mg/μg)	Test Type	Tumor model	% Inhibition	Antitumor mechanism	References
Pleurotus ostreatus	Alkaline Method	–	20 mg	in vitro	Dalton's lymphoma	75	Antitumor activity is related to activation of adaptive immune cells (B and T cells)	Devi et al., 2015
	Hot Water	WPOP-N1	400 mg	in vivo and in vitro	Sarcoma 180	58.4	WPOP-N1 induces dose-dependent activation of TNF-α and NO	Kong et al., 2014
	Hot Water	FI	30 mg	in vivo	Ehrlich	76.5	–	Facchini et al., 2014
	NH_4 Oxalate	FII				73.6		
					Sarcoma-180	85.6		
	NaOH/NaBH4	FIII				93.6		
Pleurotus eryngii	Hot Water	PEPE 1	400 μg	in vitro	HepG-2	40.04	PEPE fractions suppressed proliferation and increased release of lactate dehydrogenase (LDH) from HepG-2 cells in a dose-dependent manner.	Ma et al., 2014
		PEPE 2				60.18		
		PEPE 3				72.44		
	Hot Water	CPPS	100 μg	in vitro	HepG-2	40.76	Activation pathway of mitochondrial apoptosis.	Wu et al., 2013
		CPPS-1				55.37		
		CPPS-2				52.37		
	Hot Water	PEPw	50 mg 100 mg 200 mg	in vivo	Renca Cancer Tumor	–	The anti-tumor activity of PEPw is related to activation of the immune response	Yang et al., 2013
	Hot Water	PEP-1	100 mg	in vitro	HepG-2	–	PEP-2 has a stimulatory effect on HepG-2 cell apoptosis and induced cell cycle arrest.	Ren et al., 2016
		PEP-2						
Pleurotus djamor	Hot Water	PE1	30 mg	in vivo	Sarcoma 180	86	Tumor growth was inhibited by treatment in a dose dependent manner.	De Barba, Silveira, Piloni, Furlan, & Pinho, 2011
		PE2				85		
Pleurotus abalonus	Hot Water	PAP	400 μg	in vitro	LoVo	62.2	Inhibitory effect was observed in a dose dependent manner.	Ren et al., 2015
Pleurotus sajor-caju	Hexane/Water	PSC-hex	–	in vitro	HCT116 colorectal cancer	–	Active to apoptosis and cell cycle promoter pathways.	Finimundy et al., 2018
	Hot Water	–	–	in vitro	Laryngeal carcinoma (Hep-2) and cervical adenocarcinoma (HeLa)	–	The inhibitory effect was observed in a dose dependent manner.	Finimundy et al., 2013
	Water	PS	1,0 mg	in vitro	HeLa	60	The antiproliferative effect against HeLa cells was time dependent.	Telles et al., 2011

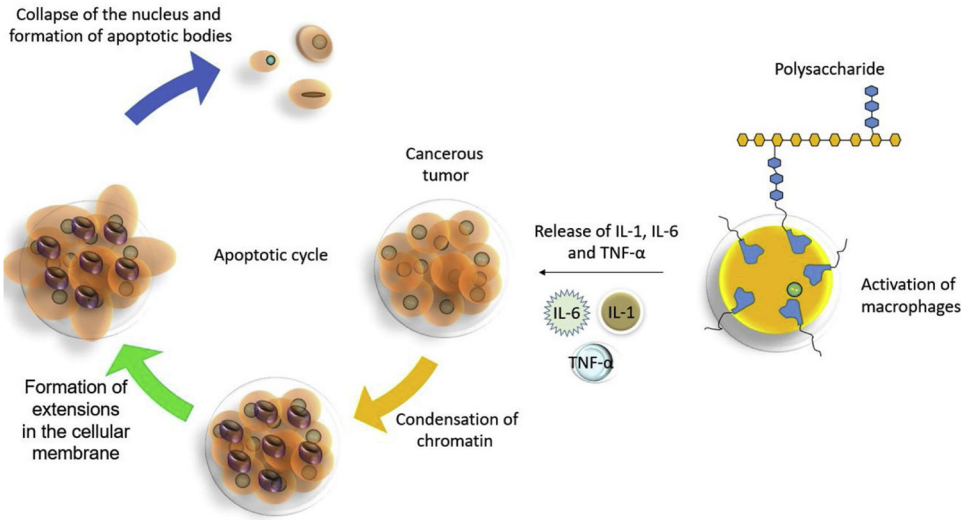

Fig. 5. Effect of polysaccharides via apoptosis induction of tumor cells.

commands the reprogrammed cell death pathway in mitochondria (Kang et al., 2015; Komada & Muruve, 2019; Park, 2018; Powley, Hughes, Cain, & Macfarlane, 2016).

Cui et al. (2014) evaluated *Pleurotus* nebrodensis polysaccharide (PN50 G) on proliferation and apoptosis of A549 cells. The PN50 G affected the proliferation viability of MRC-5 human fetal lung fibroblast cells by activation of caspase-3 and caspase-9. The qRT-PCR results revealed that caspase-3 and caspase-9 expression increased significantly in the PN50 G treated group (200 μg / mL) compared to that of the control cells. These results indicate that the expression increase of caspase contributes to PN50G-induced apoptosis of A549 cells.

It is known that the mitochondrial membrane transition pore opening is an important step for the release of cytochrome c, which binds to apoptosis protease activation factor 1 (Apaf-1), this opening leads to complex formation. multimeric, also known as apoptosome, responsible for activating pro-caspase-9 (Elena-real et al., 2018; Wang, Li et al., 2016; Wu et al., 2016). On the other hand, caspase-8 cleaves specific proteins that are transported to mitochondria, leading to cytochrome c release (Gyongyosi et al., 2019; Yang et al., 2019). Finally, caspase-3 enters the biochemical pathway as effector caspase, acting to regulate neutrophil apoptosis (Ponder & Boise, 2019). Parallel to the caspase pathway, mushroom polysaccharides of the genus *Pleurotus* spp. induce defense cells to produce nitric oxide (NO) promoting cytokine release, contributing to anticancer activity (Chen et al., 2015; Hou et al., 2019). Fig. 7 summarizes the route of action of polysaccharides by activation of the caspase pathway and depolarization of the mitochondrial membrane.

5.2. Antioxidant activity

The redox (oxidation-reduction) reaction is one of the most important reactions in living organisms. This type of chemical reaction is characterized by changes in the oxidation state of atoms due to electron transfer between two chemical species. Thus, two half-reactions occur together, one half is reduced and the other oxidized. The oxidized reaction is characterized by the loss of electrons and the increase in oxidation numbers, while the reduction reaction is characterized by the acceptance of electrons and the decrease in oxidation numbers (Meng et al., 2017). Thus, oxidizing agents accept electrons from other chemical species and are therefore reduced, thus acting as electron receptors in cellular redox reactions (Meng et al., 2017). Under normal circumstances free radicals are part of biochemical cycles and act efficiently on the mechanisms of cell growth regulation and inhibition of viruses and bactéria (Meng et al., 2017).

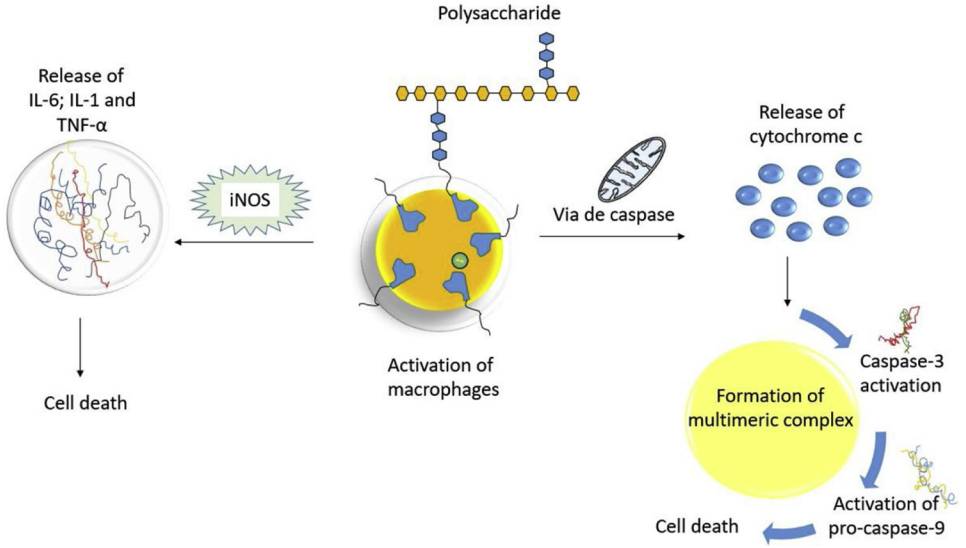

Fig. 6. Effect of polysaccharides by activation of the nitric oxide pathway.

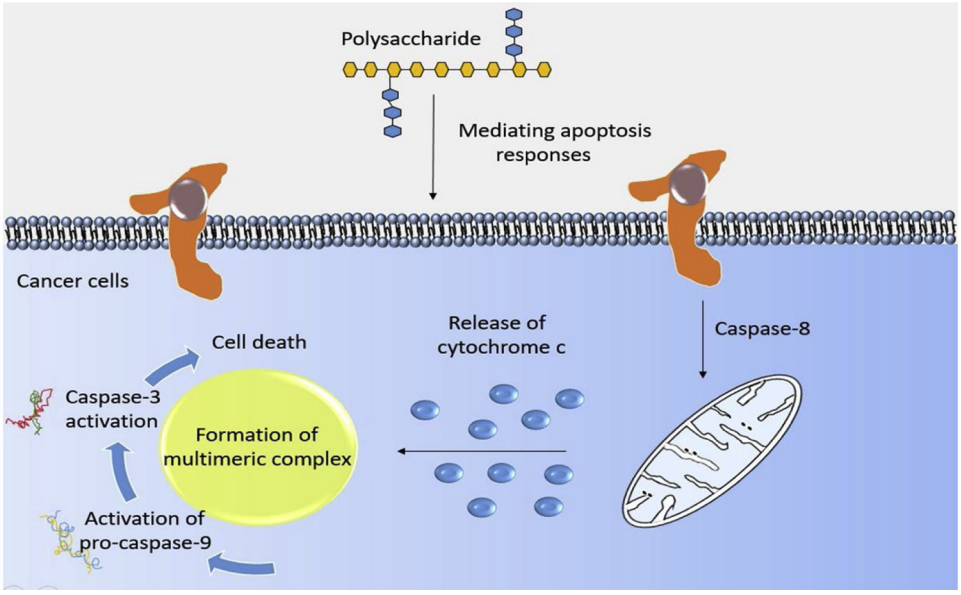

Fig. 7. Effect of polysaccharides by caspase pathway activation and mitochondrial membrane depolarization.

The increase in free radicals (oxidants) in uncontrolled cell metabolism leads to various damage to the defense cells and thus a reduction in immune function leading to cell aging, cancer, neurodegenerative and cardiovascular diseases (Chen et al., 2016). The main free radicals are those produced by reactive oxygen (ROS) and nitrogen (RNS) species, these free radicals, mainly superoxide anion, hydrogen peroxide and NO, are very toxic and reactive, are the cause of many chronic human diseases (Xie et al., 2010).

The reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide and hydroxyl radicals are produced in metabolism when cells need to produce energy, ie during respiration or even during cell differentiation and other catabolic pathways. and anabolic, reducing molecular oxygen to water and forming singlet oxygen, very reactive compound (Weydert & Cullen, 2010). Low levels of (ROS) are used by metabolism for biochemical processes of intracellular message transmission during cell differentiation and apoptosis arrest. However, high levels of reactive oxygen species (ROS) may attack tissues resulting in oxidative stress. (Weydert & Cullen, 2010).

Several natural compounds, especially secondary metabolites such as phenolic compounds and alkaloids, are excellent antioxidants. However, other metabolites such as primary metabolites, especially polysaccharides, have the ability to eliminate free radicals and may have antioxidant and anti-aging activities. Some studies (Muszyńska, Grzywacz-Kisielewska, Kała, & Gdula-Argasińska, 2018; Xiao, Chen, Li, Huang, & Fu, 2019; M. Zhu, Nie, Liang, & Wang, 2013), have already demonstrated the potential of polysaccharides in inhibiting lipid peroxidation, increasing the ability of organisms to eliminate free radicals, acting as anti-aging agents and preventing cardiovascular disease. (Xu et al., 2016), degenerative diseases like Alzheimer's (Zhonghui, Xiaowei, & Fang, 2014; Zhou et al., 2010), and against cancer (Hereher, ElFallal, Toson, Abou-Dobara, & Abdelaziz, 2018; Khan, Gani, Khanday, & Masoodi, 2018). The following Table 4, shows some mushroom polysaccharides of the genus *Pleurotus* spp. with antioxidant activity.

5.3. Immunomodulatory activity

Studies show that polysaccharides exert various biological activities indirectly through the host immune system, instead of acting with a direct cytotoxic effect (Cheng et al., 2019; Wang, Zhang et al., 2019). In this way, polysaccharides help the host to withstand adverse biological stresses from triggering chemical reactions during tumor formation eg by enhancing immunity against cancer cells by stimulating some or all biochemical defense pathways such as previously demonstrated (Vetvicka et al., 2019; Wang, Zhang et al., 2019). Thus, these polysaccharides are considered as modifiers of biological activity because they activate the innate immune system of the host, accelerating the defense mechanisms, thus exerting biological activity (Sun & Li, 2017).

The immunomodulatory activity is described as an action to improve the host's immune function by activating the adaptive immune system through stimulating factors (Chen et al., 2019; Fischer et al., 2019; Zhou, Li et al., 2019, 2019b). The adaptive immune system is also called the acquired or specific immune system. Therefore, mushroom polysaccharides of the genus *Pleurotus* spp. and other fungi in general, may increase cytotoxic activity of NK cells by improving expression of TNF-α and IFN-γ from macrophages and lymphocytes (Pushparajah et al., 2016; Zhou, Li et al., 2019, 2019b). It is already known that immunopotentiation antitumor effects can be evaluated via macrophage phagocytosis mechanisms, late-type sensitivity response or even antibody production (Liu, Kuang, Wu, Jin, & Sun, 2016; Yu et al., 2016). Specific studies on the mechanisms of polysaccharide antitumor activity revealed that these biopolymers can induce cytokine expression by a Toll-4 receptor modulated protein kinase signaling pathway (Fan et al., 2019; Hari et al., 2019; Lin et al., 2019; Xie et al., 2016).

Immunostimulatory polysaccharides can interact with the immune system both directly and indirectly, initiating a series of molecular events within the cellular microenvironment, leading to signaling and activation of the host immune system (Xie et al., 2016). Innate defense cells such as macrophages, monocytes, lymphocytes and neutrophils, as well as NK cells, are prime targets for coupling and biochemical signaling between polysaccharides and specific cell wall proteins or even within the cell (Altan-Bonnet & Mukherjee, 2019; Reis, Mastellos, Hajishengallis, & Lambris, 2019; Shaul & Fridlender, 2019). The polysaccharide immunostimulatory activity has been reported primarily from macrophage function. Recent studies corroborate that polysaccharides, especially beta-glucans (β-1 → 3,6) - D - Glcp with branching positions (β-1 → 6) - D - Glcp, activate the immune system by increasing the phagocytic function of macrophages (Bai et al., 2019; Hu, Jiang, Huang, & Sun, 2016; Maheshwari et al., 2019). In addition to the polysaccharide-stimulated phagocytic activity, other macrophage functions may be enhanced such as the production of nitric oxide via caspase pathway as discussed above, as well as increased production of reactive oxygen species (ROS), increased secretion of compounds. The proinflammatory drugs from cytokines and chemokines such as interleukins (IL-1, IL-6, IL-8, IL-12), interferon (IFN-γ), tumor necrosis factor

Table 4
Polysaccharides of *Pleurotus* spp. mushrooms with antioxidant activity.

Mushroom	Antioxidant test model	Antioxidant potential	References
Pleurotus ostreatus	DPPH	46.88%	Khan et al., 2017
	ABTS	76.87%	
	Energy reducer	66.42%	
	Ferrous ions	68.70%	
	DPPH	58.92%	Ma, Yang et al., 2018
	ABTS	62.28%	
	Energy reducer	0.636 mg/mL	
	Hydroxyl Radical	74.87%	
	DPPH	97.4%	Yan et al., 2019a, 2019b, 2019c
	Energy reducer	0.428 mg/mL	
	Hydroxyl Radical	50%	
	Superoxide Radical	90.1%	
Pleurotus eryngii	Energy reducer	0.76 mg/mL	Zhang, Li et al., 2016, 2016b; Zhang, Yang et al., 2016
	Hydroxyl Radical	66.09%	
	Hydroxyl Radical	77.29%	
	DPPH	64.90%	
	Hydrogen peroxide	93.71%	
	Ferrous ions	78.9%	
Pleutorus sajor-caju	DPPH	41%	Boonsong & Klaypradit, 2016
	Energy reducer	0.37 mg/mL	
	Radical Superóxido	25%	
	Ferrous ions	40%	
Pleurotus abalonus	DPPH	75.4%	Ren et al., 2015
	Superoxide Radical	78%	
	Superoxide Radical	75%	
Pleurotus djamor	DPPH	32.8%	Dulay et al., 2017
	DPPH	85.6%	Nattoh, Musieba, Gatebe, & Mathara, 2016
	Superoxide Radical	96%	Zhang, Li et al., 2016, 2016b; Zhang, Yang et al., 2016
	Hydroxyl Radical	65%	
	DPPH	90%	
	Energy reducer	1.9 mg/mL	

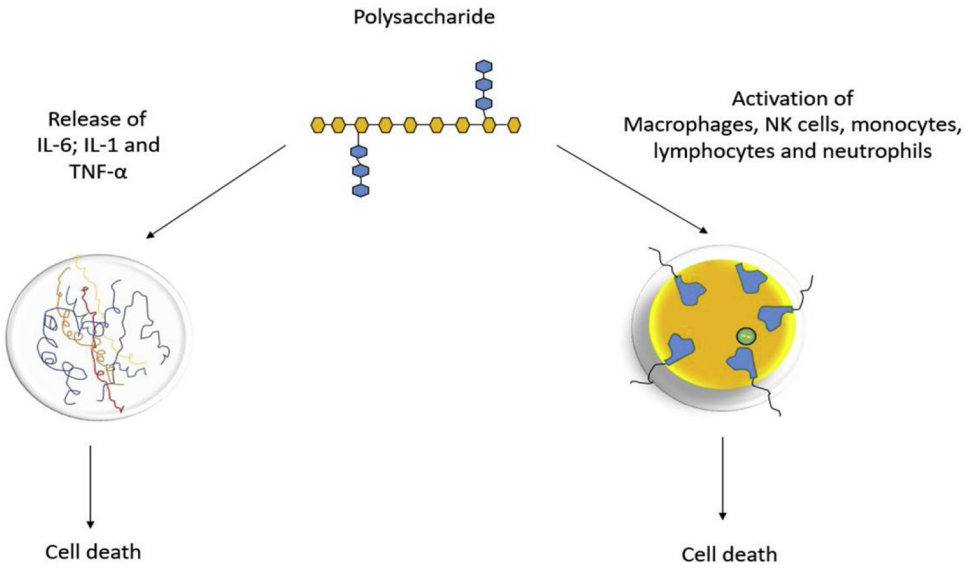

Fig. 8. Illustration of the immunostimulatory activity of the polysaccharide from the genus *Pleurotus* spp.

(TNF-α). They may also stimulate macrophage reproduction, differentiation, and cell multiplication (Ma et al., 2016; Minato, Laan, Ohara, & van Die, 2016; Telles et al., 2011). Fig. 8, below illustrates how polysaccharides can activate the immune system through various molecular events inside and outside the cellular microenvironment.

The activation of the immune system via polysaccharides is initially mediated by cell wall receptors that make specific biochemical recognition, determining the type of cellular response (Telles et al., 2011). The cell-wall receptors are called pattern recognition receptors (PRRs), and are attached to cells by extensive germline molecular cable networks that act as the biochemical information transmission network (Garaude, Kent, Van Rooijen, & Blander, 2012; Zhang, Li et al., 2016, 2016b; Zhang, Yang et al., 2016). Among these cellular receptors, some are quite relevant today, as they are the key to the development of drugs more assertive to the molecular target. Thus, the pattern recognition receptors (PRRs) found in the cell membrane are mainly Toll-like receptors (TLRS), which are responsible for binding innate and adaptive immunity, receptor scavengers (SRs), such as those of class A, β-glucans receptor, complement receptor type 3 (CR3) and mannose receptor (Dai et al., 2019; Nagarsheth, Wicha, & Zou, 2017; Di Paolo & Shayakhmetov, 2016). Other receptors, however, may be present in the bloodstream or even tissue fluids such as serum proteins, phytolins, C-

reactive protein (CRP), lipopolysaccharide binding proteins (LBP), mannan-binding lectin (MBL) and other pathway-associated proteins. alternatives, such as B and T cell antigen receptors present in lymphocytes (Balin et al., 2018; Rodríguez-jorge et al., 2019; Sng et al., 2019; Zhou, Cui et al., 2019).

6. Development of new technologies

The latest findings in the carbohydrate chemistry literature from natural sources, such as mushrooms, show that these biopolymers have interesting characteristics, which contributed to the development of new technologies. The carbohydrate biomolecules, their chemical structures, physicochemical properties, and biological potential have caught the attention of researchers who have devised new strategies to improve and enhance biological effects, or even use these biopolymers in new vaccine formulations.

The need for more effective drugs, less toxic, and with better designed bioactive has pushed chemical carbohydrate research into new horizons. Selenized polysaccharide production has been developed to improve the solubility of some polysaccharides. In addition, the technology can be used to make molecules with new chemical bonding points, which makes it possible to use these compounds for other commercial purposes as interesting sources of selenium for foods (Cheng et al., 2018).

The production of vaccines with polysaccharides as adjuvants in the formulations comes from the need to use bioactive compounds with immunoprotective potential. In addition, the production of vaccines that are more efficient, with greater safety in terms of the attenuation of microorganisms and lower toxicological risks contributes to the development of biochemical strategies for the production of vaccines with polysaccharides from mushrooms. The production of vaccines with polysaccharides is an innovative strategy from a clinical and biochemical point of view, as the potential immunoprotective and low toxicity are metabolized when the metabolism is used or the body's receptor develops innate immunity. Moreover, the source of polysaccharide is natural as it comes from microorganisms that can be cultivated with full quality and safety control (Valguarnera & Feldman, 2017). These technologies must be developed and improved for strategic reasons, which aim in the long term to improve and benefit society with new drugs and functional foods. Following, the development of these new technologies will be properly addressed and deepened.

6.1. Selenization of polysaccharides

Selenium polysaccharide conjugation has been explored in several recent mushroom reports (Li, Chen, Wang et al., 2018, 2017; Xu et al., 2016; Zhang, Li et al., 2016, 2016b; Zhang, Yang et al., 2016), plants and fruits (Lee, Lee et al., 2018; Qin et al., 2019; Ren et al., 2017; Wang, Li et al., 2016, 2016b; Zhang, Li et al., 2016, 2016b; Zhang, Yang et al., 2016), bacteria (Cheng et al., 2017; Guo et al., 2013; He, Wu, Pan, Guo, & Zeng, 2017; Xu, Wang, Jin, & Yang, 2009; Xu, Qiao, Guo, Ma, & Cheng, 2018), algae (Hao et al., 2019; Xiao et al., 2019), and in bibliographic review papers (Cheng et al., 2018; Li et al., 2019; Xu et al., 2019), which highlight the potential synergistic effect between polysaccharide and selenium, selenization methods, their correlation as dietary supplement, selenium source and their bioactivities including antioxidant, hepatoprotective, neuroprotective, antitumor, antidiabetic and anti-inflammatory activities (Chen et al., 2019; Devi, Behera, Mishra, & Maiti, 2015; Hamid et al., 2017; Hou et al., 2019; Liu, Zhu, Sun, Gao, & Zhang, 2017).

Selenium (Se), is an important micronutrient responsible for several physiological processes essential for humans, being remedied by a complex of enzymes specialized in selenium metabolism (Wei et al., 2019). The nutritional requirement of selenium is due to the fact that most foods are low in this element. Selenium deficiency in diet can lead to necrotic liver degeneration, cardiovascular disease, cancers, aging, mortality from infectious and chronic diseases, and influence the formation of various enzymes with antioxidant catalytic activity (Malinowska et al., 2018; Zhang, Song, Ng, Zhao, & Liu, 2013).

Selenium is an important cofactor in the formation of several antioxidant enzymes, such as glutathione peroxidase (GSH-Px), superoxide disputasse (SOD) and thioredoxin refutasse (TRx) (Phiri et al., 2019). These enzymes are important for maintaining various protective cycles of cellular metabolism. When they are in their reduced state, they are responsible for catalyzing the breakdown of lipid hydroxides and hydrogen peroxides within human cells, protecting cells and the cell membrane from oxidative damage (Peng, Lin, Xu, & Zhang, 2016; Shanshan et al., 2018; Tugarova & Kamnev, 2017).

Selenium-containing compounds can be divided into two groups based on the type of selenium speciation, so we have selenium-like inorganic compounds (selenites and selenates), and organic compounds that include (selenium-containing polysaccharides, selenoproteins and selenoamino acids). When these two groups are compared, it is observed that selenium-containing organic compounds have several advantages, such as higher bioavailability and lower toxicity, and several bioactivities enhanced by the binding of these metabolites with selenium (Li et al., 2019; Xu et al., 2019).

In recent years some work has explored the potential of selenium to modify polysaccharide bioactivities, as previously described, several mushrooms, bacteria, algae and plants have been tested for this purpose, however this technology is still under development when exploited for mushrooms of the genus *Pleurotus* spp. Recent work demonstrating that selenium polysaccharides from biotechnology with *Pleurotus* spp. are of great potential due to recent discoveries of bioactivity and their relationship to polysaccharide structure as well as their relationship to molecular structures modified by the addition of selenium atoms (Xu et al., 2019).

The physicochemical properties of polysaccharides, as well as molecular weight, structural conformation and branching, are altered by the presence of selenium atoms. The effect of the addition of selenium atoms to the polysaccharide structure contributes to the increase of solubility of these polymers in aqueous medium, enhancing the diffusion efficiency in the extracellular medium and therefore their binding with cell wall receptors (Li et al., 2019). Selenium-containing polysaccharides are promising in the fight against various diseases, as structure modifications are sufficient to enhance existing bioactivities, so recent work has explored selenization technology, emphasizing structure preparation, molecular characterization and the biological of potential evaluation (Cheng et al., 2018).

The mushroom cultivation on substrates supplemented with selenium salts is an interesting strategy for producing organic selenium enriched foods. Thus, the edible mushroom *Pleurotus* florida was properly cultivated in residues of wheat straw supplemented with inorganic selenium, and the potential for accumulation of this microelement from the cultivation substrate was evaluated. In this study, it was found that the selenium concentration in the mushrooms was 800 times higher when compared to the control, and the results of HPLC-ICP-MS revealed that selenium was in the form of selenoamino acids and also bound to cell wall polysaccharides. This approach can be used for bioconversion of inorganic selenium to organic selenium with the potential nutritional and therapeutic act (Bhatia et al., 2013). Another study with the *Pleurotus* ostreatus grown in selenium-supplemented coffee residues has shown that these mushrooms are efficient microelement bio accumulators and are therefore important means of selenium enrichment for food (Marliane et al., 2012).

Most recent studies with the mushroom *Pleurotus* ostreatus has focused on evaluating the potential pharmacological effects of cell wall polysaccharides (Milovanovic et al., 2019). However, few studies have examined the biological effects of selenium-bound polysaccharides, the so-called Se-POP at the cellular level. In a recent study, the mushroom *Pleurotus* ostreatus was grown in a mixed medium containing sawdust, wheat bran, cornmeal, plaster and supplemented with sodium selenite

(Na_2SeO_3). Selenium-containing polysaccharides (Se-POP) were extracted, structurally characterized, and their antioxidant and protective effects were evaluated against H_2O_2-induced cytotoxicity in cell media. Se-POP has been described as a heteropolysaccharide with an average molecular weight of 0.95×10^4 Da. This polysaccharide showed antioxidant activity in models such as ABTS, DPPH, hydroxyl radicals and in vivo models against H_2O_2-induced oxidative stress. The results suggest that Se-POP has antioxidant properties in vitro and in vivo and can be exploited as a food ingredient or as a medicine (Xu et al., 2016).

Another paper, with the mushroom *Pleurotus* ostreatus and *Pleurotus* djamor cultivated in selenium supplemented rice straw residues with the Se (IV), showed that these mushrooms are efficient selenium bioaccumulators, and that the presence of this microelement in proteins such as prolamins, glutelins, globulins and polysaccharides increases the bioaccessibility of other microelements such as Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn. Therefore, selenium accumulation was important for the formation of selenium-containing selenoproteins and polysaccharides, as well as helping in the bioaccessibility of other microelements (De Oliveira & Naozuka, 2019).

A selenium enriched polysaccharide (SE-) SPMP-2a was also extracted, purified and characterized from the mushroom *Pleurotus* geesteranus. The polysaccharide is white, flocculent and water-soluble, described as a α-D-glycopyranoside-linked Se-acid polysaccharide, average molecular weight of 3.32×10^4 Da. Effects against H_2O_2-induced oxidative stress were evaluated in cells. HaCaT. The addition of SPMP-2a to oxidative stress cell media improved cell viability, nuclear condensation decreased and cell apoptotic rates significantly reduced. SPMP-2a has been found to activate B cell lymphoma (Bcl-2) protein expression, improving antioxidant enzymatic activity by reducing ROS as well as cellular apoptosis (Sun, Zhou, Huang, & Jiang, 2017).

6.2. Vaccine production

Vaccination is one of the triumphs of modern medicine, as it represents a leap in knowledge and technology against various diseases that could not otherwise be avoided. However, vaccine exploitation is currently limited by a number of issues such as lack of in-depth knowledge of immunoprotective antigens, safety of attenuation of microorganisms, limitation of vaccination of immunocompromised individuals, high production costs and low coverage (Finn, 2018; Hollingsworth & Jansen, 2019). Research into alternative vaccine production platforms has been explored to replace dead or attenuated microorganism platforms with safer and more suitable sources for delivery (Kay, Cuccui, & Wren, 2019; Pardi, Hogan, Porter, & Weissman, 2018).

Currently, a considerable number of publications (Micoli, Costantino, & Adamo, 2018; Rappuoli, 2018; Valguarnera & Feldman, 2017; Weyant, Mills, & DeLisa, 2018), It has been shown that some hosts may be used for vaccine subunit production. These vaccine production platforms consist of inexpensive low-processing biomass sources, ideal for the formulation of economical and efficient oral vaccines. Among these new platforms the most recent include fungi (Han et al., 2019; Liu, Zhou et al., 2016; Remondo et al., 2009; Zhang et al., 2011), microalgae (Pérez-Martínez, Acevedo-Padilla, Bibbins-Martínez, Galván-Alonso, & Rosales-Mendoza, 2015), bacteria (Amuguni, Tzipori, Amuguni, & Tzipori, 2012; Pérez-Martínez et al., 2015) and plant cells (Rosales & Salazar, 2014). These platforms are recognized as safe because they come from edible biomass which enables the production of oral vaccines with minimal biomass processing, reducing production and formulation costs (Micoli et al., 2018).

The use of edible fungi, mainly yeast and basidiomycetes, has been explored for vaccine production and delivery, as they have several attractive characteristics. The mushrooms of the genus *Pleurotus* spp., have several desirable characteristics for vaccine production and delivery, such as edible biomass, low production cost, short growing periods, large scale production in bioreactors, process parameter controls and production of various compounds (proteins, polysaccharides and polysaccharides), with immunomodulatory activities, as well as the availability of various methods of transformation and genetic improvement (Sun, Pan et al., 2017).

Three important aspects must be considered for the production and delivery of vaccines from genus mushrooms *Pleurotus* spp. Primeiramente, vaccine production depends on efficient platforms, so genetic engineering approaches are sometimes required to generate a sufficient number of high expression clones with unchanged phenotype. Several methods and approach are reported in the literature, as it is not the focus of this paper to emphasize these aspects, the following papers can be consulted for further clarification (Kim, Sapkota, Choi, & Kim, 2010; Pérez-Martínez et al., 2015; Wang, Zhao et al., 2018).

The second relevant aspect in choosing suitable hosts for biopharmaceutical production is related to the post-translational modification pathways, in particular the protein glycosylation pathway (Sun, Pan et al., 2017). This pathway is important because it influences half-life, molecular bioaffinity between target and drug, and influences immunological properties (Kay et al., 2019). For further information, the following articles may be consulted (Kang, Park, Lee, Yoo, & Hwang, 2018; Kay et al., 2019; Wild et al., 2018), where glycosylation mechanisms are widely discussed in model organisms.

Finally, vaccine production platforms should synthesize immunomodulatory compounds, in this regard, it is noteworthy that mushroom polysaccharides of the genus *Pleurotus* spp., have immunomodulatory activity, as described in a previous topic. In particular bioactive mushroom compounds of the genus *Pleurotus* spp. include more than polysaccharides as lectins used as adjuvants for DNA vaccine production (Martin et al., 2016). Others as terpenes with antioxidant, anti-inflammatory and immunomodulatory activity (Silva et al., 2012), flavonoids (He, Tan, Liu, & Zhao, 2019) and glycoprotein complexes such as α-glucan-protein with proinflammatory and immunomodulatory activity (Atitmanwiwat et al., 2012).

The potential of β-glucans as an adjuvant in vaccines and other drugs has already been properly evaluated in other microorganisms, providing much information on the mechanisms and challenges of this new technology. In this regard, p-glucan particles (GPs) have been used as a delivery vehicle for drugs and antigens, as these biopolymers can be recognized by cell membrane receptors, thus bringing drugs into the cellular environment and delivering them within. of the cell (Huang, Ostroff, Lee, Specht, & Levitz, 2013). In addition, p-glucan particles (GPs) can encapsulate and carry a variety of molecules such as DNA, proteins, RNA and enzymes, acting as a targeted, controlled and personalized vaccine delivery platform (De Jesus, Ostroff, Levitz, Bartling, & Mantis, 2014).

Few reports are available on the use of mushrooms *Pleurotus* spp., as a platform for vaccine production. However, some relevant work on vaccine production will be discussed. β-glucan has been evaluated as an adjuvant in puppy vaccination against canine parvovirus and rabies virus. The administration of β-glucan as an adjuvant was found to increase the activity of the innate immune system by activating leukocyte phagocytic pathways, polymorphonuclear chemotactic activity and lymphocyte blastogenic response (Aladová, Ojžišová, Peter, Ndrejková, & Ojtek, 2011).

Hepatitis B is a chronic infection, a serious problem, even with current vaccines the problem still affects millions of people worldwide. Currently, the DNA vaccine strategy has been considered promising to activate immune responses against infection. In order to increase the immunogenicity of the HBV DNA vaccine, the purified lectin polysaccharide from *Pleurotus* ostreatus (POL), was tested as an adjuvant to the vaccine in trials with transgenic C57BL/6 and HBV surface antigen mice (HBVsAg-Tg). The results revealed that low concentrations of POL were sufficient to activate a more efficient stimulant response than just the vaccine. Polysaccharide from *Pleurotus* ostreatus (POL) activated the immune response of cells such as lymphocytes (Th2 and Tc1), increasing the surface-specific protein antibody (HBVsAb), thereby

effectively stimulating the immune system (Gao et al., 2013).

7. Concluding remarks

Polysaccharides of mushroom from the genus *Pleurotus* spp., are important biological structures, responsible for several bioactivities with scientific relevance and profound impacts on society, economy, income generation, and technology development. It was observed that this genus has many peculiarities, which contributes to its biodiversity, taxonomic, sexual characteristics and biochemical composition. Recent advances in extraction techniques are increasing extraction yields, sequentially improving separation conditions, leaving the purest strata free of interferents and contaminants.

The bioactivities related to these biopolymers were addressed on the mechanistic aspect of the action. It has been demonstrated how polysaccharides act through several biochemical routes, besides the characteristics related to ligand proteins, receptors, and mechanisms of indirect action by the activation of the host immune system. To finalize the new technologies approached emphasized the production of selenized polysaccharides and production of vaccines. The question was raised about the importance of these technologies and their impacts on the structure, conformation, and bioactivity of polysaccharides. Mushroom vaccine production has been raised, and its mechanisms have been addressed, the possibility of technology being improved has been questioned, taking into account the need for new, cheaper, less expensive, and less invasive platforms. Finally, much remains to be explored, taking into account the biodiversity of mushrooms from the genus *Pleurotus* spp., and biotechnological possibilities, cloning, production of somatic hybrids and development of new polysaccharide extraction tools. Only the future can reveal all the contributions, but so far the story told with the mushrooms of the genus *Pleurotus* spp. is a success for the advancement of science, technology, and innovation.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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CAPÍTULO II

Obtaining extracts rich in antioxidant polysaccharides from the edible mushroom *Pleurotus ostreatus* using binary system with hot water and supercritical CO_2

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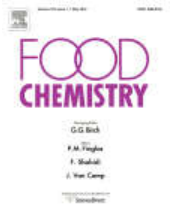

Obtaining extracts rich in antioxidant polysaccharides from the edible mushroom Pleurotus ostreatus using binary system with hot water and supercritical CO2

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ABSTRACT

Pleurotus ostreatus is an edible mushroom with pharmacological potential, due to its metabolites, mainly polysaccharides. On here, the development of a new methodology for the recovery of extract rich in antioxidant polysaccharide was reported. The extracts were characterized, evaluated for antioxidant activity *in vitro* and in cell models and cytotoxicity. The best defined extraction condition was 25 MPa, 433.15 K, and 20% H_2O, with 30.69% of the total yield and 0.921 mg of CHO_3. The anomeric bonds, identified in the FTIR and NMR spectrum, indicate that the extracts are a mixture of heteropolysaccharides, β-glucans, α-glucans, and oligosaccharides. The best extraction condition has 80.83% of antioxidant activity, without cytotoxic effect *in vitro*. In addition to antioxidant activity in cell model, increasing protection against oxidative damage induced by H_2O_2. Finally, H_2O + CO_2-SFE technology can be used to obtain extracts rich in antioxidant polysaccharides with pharmacological and food potential.

1. Introduction

Edible mushrooms are very important for culinary and for the production of metabolites of interest. *Pleurotus ostreatus* better known as oyster mushroom is one of the most produced and consumed mushrooms in the world. It has been used in the formulation of several gastronomic dishes, it is very nutritious, it has a pleasant flavor and aroma, in addition to important vitamins, minerals and metabolites such as polysaccharide and glycoprotein complexes (Bellettini et al., 2019).

The polysaccharides are one of the most studied metabolites of the species *Pleurotus ostreatus*, extracted from fruiting bodies, mycelium, sclerotia and broth after fermentation. Polysaccharides and glycoprotein complexes play important roles along the fungal cell wall, such as cell-structuring molecules, pH modifiers, ligands, and molecular receptors, biosynthesis target sites, and nutrient transport. In addition, these biopolymers have important bioactivities, such as anti-tumor (Chen & Xue, 2019), antioxidant, anti-inflammatories, hypocholesterolemic (Gil-Ramírez, Morales & Soler-Rivas, 2018) and immunostimulator (Sun & Li, 2017).

Abbreviations: $H_2O + CO_2$-SFE, Extraction with hot water and addition of supercritical carbon dioxide; POP, *Pleurotus ostreatus* polysaccharide; POP-SFE, *Pleurotus ostreatus* polysaccharide extracted by supercritical fluid; CHO_3, Equivalent in polysaccharides; MSR, Response Surface Methodology; wb, Wet basis; POP-SFE-D, *Pleurotus ostreatus* deproteinizated polysaccharide extracted by supercritical fluid; POP-SFE-P, Crude extract, containing polysaccharides and proteins

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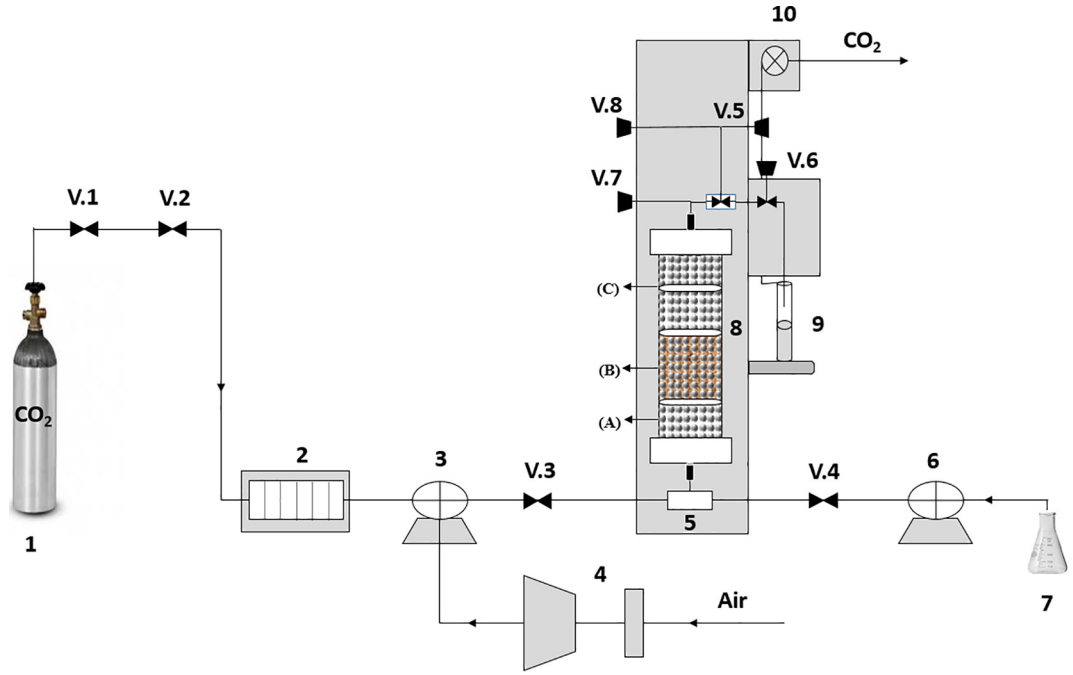

Fig. 1. Schematic of a supercritical fluid extraction plant applying solvent/cosolvent. CO_2 cylinder (1); cooling bath (2); booster; CO_2 pump (3), Compressor (4); mixer (5); Solvent Pump (6); cosolvent receptor (7); extraction unit and extraction bed (8); control valve (V.1 to V.8); separation container (9); flow meter (10). The extraction bed (8). A) Glass spheres. B) Cotton. C) Glass spheres + sample.

The polysaccharides are normally extracted by hot water extraction technique (Wang, Cao, Zhang, & Chen, 2019), microwave-assisted extraction (Hu et al., 2019) acid and alkaline extraction (Gao et al., 2019) and ultrasound-assisted extraction (Morales et al., 2019). However, these techniques have some disadvantages such as low extraction yield, long extraction time and the need for several extractions to obtain considerable amount of crude polysaccharides. Given this scenario, the technology of supercritical fluid and water as cosolvent (H_2O + CO_2–SFE) may be a viable alternative for polysaccharide recovery. Recent work on biomass sugar recovery and polysaccharide extraction using pressurized liquids (Gallego, Bueno, & Herrero, 2019), help in the development of experimental design with controlled temperatures and pressures that can be used and modified for polysaccharide recovery without hydrolysis of these compounds. According to Benito-Román et al. (Benito-Román, Alonso, Cocero, & Goto, 2016), this technology can be applied for mushroom β-glucan extraction. Temperatures between 120–180 and pressure up to 400 bar, improve polysaccharide extraction, in addition to the addition of supercritical CO_2, which helps in acidification of the medium and mass transport.

In this work, the development of a new methodology for the recovery of an extract rich in antioxidant polysaccharide obtained from the edible mushroom *Pleurotus ostreatus* using hot water with the addition of supercritical carbon dioxide (H_2O + CO_2-SFE) was reported. The trend lines that show which conditions are suitable for extraction were made using the Box-Behnken statistical model. Extracts rich in polysaccharides were pre-purified, characterized, and evaluated for antioxidant activity *in vitro* and in a cell model, in addition to cytotoxicity.

2. Materials and methods

2.1. Materials and reagents

The fruiting bodies of the mushroom *Pleurotus ostreatus* (2 kg with 89% of moisture) were purchased from a certified supplier MNS Group (São Paulo, Brazil). After storage at 4 °C, the fresh mushroom was ground at 2500 rpm for 1 min in a blender (PH900 Jarra, Philco, Brazil). Actual particle density was determined using the Archimedes principle (Loverude, Kautz, & Heron, 2003). The apparent density of the bed was calculated by the ratio between the mass of *Pleurotus ostreatus* and the volume of the extraction bed. The bed porosity was calculated using the mathematical relationship of the actual and apparent densities (Gallego et al., 2019). Glucose (> 98%) was used as a standard for the construction of the calibration curve for total sugars, it was purchased at LABSYNTH Laboratory Products Ltd. (São Paulo, Brazil). DPPH (1.1-diphenyl-2-picryl-hydrazil) (> 97.0%), ethanol (> 98%), phenol (> 98%), *n*-butanol (> 98%) and chloroform (> 98%), were purchased from ISOFAR Chemical Industry and Commerce Ltd. (Rio de Janeiro, Brazil). DEMEN culture medium and the aprotic solvent DMSO were purchased from SINAPSE Biotechnology Ltd. (São Paulo, Brazil). HepG2 cells (hepatocarcinoma) were obtained from the cell bank of Rio de Janeiro (BCRJ). Carbon dioxide CO_2 was purchased from White Martins (Belém, Brazil).

2.2. Conventional extraction

Extraction was performed with distilled water at 100 °C over a period of 3 h, and the ratio of raw material to water was 1:20, w/v. The extracts were recovered and separated from the biomass by filtration, concentrated under vacuum at 60 °C and stored cold at 4 °C for later polysaccharide recovery (POP).

2.3. Supercritical extraction unit

Extractions were performed at the Supercritical Extraction Laboratory (LABEX/UFPA/Brazil) in a SPE-ED SFE unit (model 7071, Applied Separations, USA), coupled to a CO_2 cylinder, a compressor (model CSA 78, Schulz S/A, Brazil), recirculator (model F08400796, Polyscience, USA) and a CO_2 flow meter (model M 5SLPM, Alicat Scientific, USA). The extraction cell used has an internal height of 0.323 m and an internal diameter of 0.0142 m. The densities of the binary system (H_2O + CO_2-SFE) were calculated using the Aspen Hysys software (Aspen One 8.6), applying the Peng-Robinson cubic state equation, with null binary interaction parameters (Peng & Robinson,

Table 1
Box-Behnken experimental design, with independent variables on the response variables evaluated for *Pleurotus ostreatus* polysaccharide extracts obtained by H_2O + CO_2-SFE.

Assays	Independent variables (original and coded)			Responses		ρCO_2 + H_2O (kg/m^3)
	PRE (X_1 MPa)	TE (X_2 K)	COS (X_3 %)	Y_1	Y_2	
1	15 (−1)	393.15 (−1)	15 (0)	3.40 ± 0.01	0.942 ± 0.07	316.9
2	35 (+1)	393.15 (−1)	15 (0)	4.12 ± 0.03	0.684 ± 0.02	698.2
3	15 (−1)	433.15 (+1)	15 (0)	28.23 ± 0.78	0.672 ± 0.10	242.1
4	35 (+1)	433,15 (+1)	15 (0)	30,73 ± 1.18	0.587 ± 0.09	572.9
5	15 (−1)	413.15 (0)	10 (−1)	3.72 ± 0.11	1.067 ± 0.11	265.6
6	35 (+1)	413.15 (0)	10 (−1)	2.44 ± 0.04	0.841 ± 0.07	469.6
7	15 (−1)	413.15 (0)	20 (+1)	3.37 ± 0.01	0.907 ± 0.12	289.5
8	35 (+1)	413.15 (0)	20 (+1)	3.64 ± 0.03	0.687 ± 0.04	649.8
9	25 (0)	393.15 (−1)	10 (−1)	2.06 ± 0.03	1.063 ± 0.06	537.7
10	25 (0)	433.15 (+1)	10 (−1)	13.55 ± 0.03	0.847 ± 0.04	415.9
11	25 (0)	393.15 (−1)	20 (+1)	2.57 ± 0.03	1.227 ± 0.04	566.6
12	25 (0)	433.15 (+1)	20 (+1)	30.63 ± 2.18	0.954 ± 0.02	444.5
13	25 (0)	413.15 (0)	15 (0)	3.68 ± 0.01	0.738 ± 0.02	483.8
14	25 (0)	413.15 (0)	15 (0)	3.58 ± 0.08	0.744 ± 0.02	483.8
15	25 (0)	413.15 (0)	15 (0)	3.32 ± 0.08	0.755 ± 0.09	483.8

PRE Pressure, TE Extraction Temperature, COS Percent Co-solvent, Y_1 Overall Yield (%), Y_2 Total Polysaccharide (CHO_3), in equiva. Glc, mg/g extract.

1976). In the (Fig. 1), a general scheme of the supercritical fluid extraction unit without solvent recycling is shown.

2.4. Obtaining polysaccharide extract with H_2O + CO_2-SFE

To obtain the polysaccharide extract, 10 g of crushed *Pleurotus ostreatus* were mixed with 20 g of glass spheres. A 1: 2 ratio (mushroom/glass spheres, w/w) was used, as reported in supplementary material (S1). Extractions were carried out at temperatures from 393.15 to 433.15 K, pressures from 15 to 35 MPa and 10 to 20% H_2O, with a flow rate of 2.5 L/min^{-1} of CO_2, as shown in (Table 1), with H_2O + CO_2-SFE was performed according to the following experimental procedure: First the system operating temperature was adjusted with the closed V.3, V.4 and V.5 valves (Fig. 1); The system was then opened and the operating pressure adjusted. After the system reached operating pressure, the dynamic extraction period (open system) of 120 min began. At the end of the extraction extracts were collected and stored cold at 4° C for later polysaccharide recovery (POP-SFE). The best extraction conditions were determined from a Box Behnken experimental design, where pressure (X_1, MPa), extraction temperature (X_2, °C) and percentage of cosolvent (X_3, %) were used as independent variables. The responses evaluated were overall yield (Y1, %) and total polysaccharides (mg CHO_3/g sample). The desired characteristics were maximum overall yield and total CHO_3. Assays were randomized to minimize the effect of external factors. The linear, quadratic and interaction effects of the independent variables in the selected response can be observed in Eq. (1). Where Y is the dependent variable, β_0 is the constant, β_i, β_{ii} and β_{iii} are the coefficients of regession and X_i and X_j are the levels of the independent variables.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta iXi + \sum_{i=1}^{k} \beta iiXi^2 + \sum_{i=1} \sum_{j=i+1}^{k} \beta iiXiXj + \varepsilon Y = f(X1,)$$
$$= \beta_0 + \beta_1(A) + \beta_{11}(A)^2 + \beta_2(B) + \beta_{22}(B)^2 + \beta_3(C) + \beta_{33}(C)^2 + \beta_4 (D) + \beta_{44}(D)^2 + \beta_{12}(AB) + \beta_{23}(BC) + \beta_{13}(AC) + \beta_{14}(AD) + \beta_{24} (BD) + \beta_{34}(CD) \quad (1)$$

The models were evaluated by the F test for regression and lack of fit, as well as analysis of variance (ANOVA) and correlation coefficient (R^2). After the evaluation of the models, only the significant variables ($p < 0.05$) were maintained from the adjusted models, the Response Surface (MSR) was constructed and the behavior was analyzed. The ideal level of each response was defined in conjunction with the desirability function. These analyses were performed using the Statistica Kernel Release 7.1 software (StatSoft Inc. 2006, Tulsa, OK, USA). The overall yield was calculated on wet basis (wb), from the mathematical relationship between the mass of the precipitated extract ($m_{extract}$) and the mass of fresh raw material fed into the extraction cell, according to Eq. (2).

$$X_{0\,wb}(\%) = \frac{m_{extrat}}{m_{raw\ material}} \times 100 \quad (2)$$

2.5. Polysaccharide precipitation, deproteinization and quantification

The concentrated extracts from conventional extraction and H_2O + CO_2-FSE extractions were used for polysaccharide recovery. The extracts were precipitated using 3 volumes of 95% ethanol at 4° C for 24 h. After centrifugation (4000 rpm, 20 min), the precipitate was collected, washed several times with ethanol and acetone to remove impurities, allowed dry the ethanol at 40° C for 20 min, then the samples were weighed and the overall yield was calculated on wet basis (wb). Fractions with better yield were deproteinized (pre-purification) by the Sevag reagent method (*n*-butanol/chloroform, 1: 5v/v), for the analysis of polysaccharides without interferences. The sugar content was detected using the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.6. Fourier transform infrared spectroscopy

The polysaccharides were ground with KBr powder and then pressed into a 1 mm pellet for Fourier transform infrared (FT-IR) measurements. FT-IR spectra were obtained using a spectrometer (Spectrum Two FT-IR) in the range of 4000 to 400 cm^{-1}.

2.7. X-ray diffraction analysis

The samples were placed in thin-walled capsules (0.5 mm outside diameter), placed in a vacuum flat film X-ray camera mounted on a 30 kV and 20 mA X-ray generator (Philips 1720) with α CuK radiation, Ni-filtered. The resulting X-ray diffraction diagrams were recorded in and processed.

2.8. Nuclear magnetic resonance (NMR) spectroscopy

NMR spectra (^{1}H) were obtained using a 400 MHz Bruker Avance III spectrometer. The analyses were performed at 70 °C in Me2SO-d6, and the chemical shifts are expressed in δ (ppm) relative to Me_2SO-d_6 and δ

2.40 (^{1}H).

2.9. DPPH radical scavenging activity

The DPPH elimination capacities of POP and POP-SFE were determined according to the method described by Ma, Zhao, Yu, Ji, and Liu (2018). Briefly, 2 mL of 2 × 10^{-4} mol/L DPPH in methanol solution was added to 2 mL of polysaccharide samples at concentrations (15 mg/mL). The mixture was vigorously stirred and left at room temperature for 30 min in the dark. Absorbance was measured at 517 nm. Vitamin C, in deionized water solution, was used as a positive control. All tests were performed in triplicate, and radical scavenging activity was calculated according to Eq. (3).

$$Scavenging\ rate\ \% = 1 - \frac{A_1 - A_2}{A_0} x100 \quad (3)$$

where A_0 is the absorbance of the blank sample, A_1 is the absorbance obtained from the sample and A_2 is the absorbance of the blank reagent.

2.10. Cell cultures and assays MTT for cytotoxic effect

The cells were grown in 75 cm^2 flasks in DMEM-type culture medium (Eagle's medium modified by Dulbecco, Gibco) enriched with fetal bovine serum (10%) at 37 °C in an atmosphere containing 5% CO_2. A density of 6.7 × 10^3 cells was seeded in each well of 96-well microplates and incubated for 24 h. Different concentrations of POP-SFE (400, 200, 100, 50 and 10 μg/ml) were added to the wells containing the HepG2 cells. After 24 and 48 h of exposure, the medium containing the extract was removed and 100 μl of MTT {[3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium]} (0.5 mg/ml) was added in each well for a period of 3 h. The solution containing MTT was removed and 100 μl of DMSO (dimethylsulfoxide) was added for dilution of formazan crystals. Finally, absorbance was measured on a spectrophotometer (BioTeK, NY, USA) with a wavelength of 570 nm, converted into a cell viability rate and expressed as a percentage (Xiong, Li, Chen, Chen, & Huang, 2016).

2.11. Evaluation of the antioxidant effect of POP-SFE against oxidative damage induced by H_2O_2

HepG2 cells were treated with different concentrations of POP-SFE (200, 100, 50, 25 and 10 μg/ml) solubilized in DMSO (0.1%) for 24 and 48 h. After the exposure time, all groups were exposed to 10 μM H_2O_2 for 2 h. The cell viability assessment (MTT) assay was the same as previously presented. The absorbance of each well was detected at a wavelength of 570 nm and converted into a cell viability rate expressed as a percentage (Xiong et al., 2016).

2.12. Statistical analysis

The other data were submitted to analysis of variance (ANOVA) and means were compared by Tukey test using Statistica Kernel Release 7.1 software (StatSoft Inc. 2006, Tulsa, USA).

3. Results and discussion

3.1. Obtaining polysaccharide extract with H_2O + CO_2-SFE

3.1.1. Overall yield

The overall yield of the extracts obtained by H_2O + CO_2-SFE ranged from (2.06% ± 0.03 wb) to (30.73% ± 1.18 wb). Higher yields do not differ significantly ($p > 0.05$) from each other, the same effect is also observed for lower yields (Table. 1). The highest overall yields (30.73% ± 1.18; 30.63% ± 2.18 and 28.23% 0.78 wb), respectively, were obtained at a temperature of 433.15 K with wide pressure range and cosolvent. On the other hand, the lowest yields were obtained at temperatures below 413.15 K, with wide pressure range and cosolvent. The higher overall yields obtained by H_2O + CO_2-SFE when compared to conventional extraction are higher. Conventional hot water extraction yielded a maximum yield of (6.21% wb/ ± 0.07% wb), while extraction by H_2O + CO_2-SFE yielded (30.73% ± 1.18 wb). The extraction carried out with hot water and supercritical carbon dioxide (H_2O + CO_2-SFE) is more efficient compared to conventional extraction, due to the high temperatures, better diffusivity of the solvent, bed porosity and drag of the solute through supercritical CO_2.

The coefficients of regression of the independent variables as a function of response (overall yield) for H_2O + CO_2-SFE are listed in supplementary material (S2). It is observed that pressure, temperature and percentage of H_2O influenced in a significant way ($p \leq 0.05$) the responses in its linear, quadratic and interaction form. The coefficient of determination (R^2) was 0.91, indicating that 91% of the response variable can be explained by the model. The lack of adjustment was significant, indicating that the model does not relate exactly to experimental data; however, it can be used to evaluate response behavior associated with high R^2 (≥0.90).

The results of the analysis of variance (ANOVA) for overall yield can be found in the supplementary material (S3). Similar results were observed in the extraction of other polysaccharides by H_2O + CO_2–SFE, as for the mushroom *Ganoderma lucidum* (Benito-Román et al., 2016), and by pressurized hot water (PHW) for waxy barley (Benito-román, Alvarez, Alonso, Cocero, & Saldaña, 2015) and *Pleurotus ostreatus* (Smiderle et al., 2017).

3.1.2. Composition in polysaccharide

The polysaccharide composition ranged from (0.587 equiv. Glc, mg/g strat ± 0.09 wb) to (1227 equiv. Glc, mg/g strat ± 0.04 wb). The highest polysaccharide composition was in assay 11 (25 MPa, 393.15 K, and 20% H_2O), being statistically different ($p > 0.05$) from the other experiments (Table. 1). However, the same experiment obtained an overall yield of (2.57% ± 0.03 wb). The lowest polysaccharide composition was in assay 4 (35 MPa, 433.15 K, and 15% H_2O), with overall yield (30.73% ± 1.18 wb). The regression coefficients of the independent variables as a function of the response (polysaccharide composition) for H_2O + CO_2-SFE are listed in the supplementary material (S2). It can be seen that pressure and temperature were not significantly influenced ($p \leq 0.05$) in the response in its linear, quadratic, and interactional form. The percentage of co-solvent does not significantly influence ($p \leq 0.05$) the response in its linear, quadratic, and interaction form. The determination coefficient (R^2) was 0.48, indicating that 48% of the response variable can be explained by the model. The lack of adjustment was significant, indicating that the model is not exactly related to the experimental data. That is, the polynomial model fails to clearly describe the phenomena that occur. The results of the analysis of variance (ANOVA) for the composition of polysaccharides can be found in the supplementary material (S3).

The results suggest that pressure and temperature may influence the polysaccharide composition in two ways. Increasing pressure along with temperature suggests a reduction in polysaccharide composition, probably due to increased extraction of soluble proteins and sample compression, preventing internal diffusion of water molecules among solid particles. On the other hand, at lower pressures and higher temperatures, an increase in polysaccharide composition is observed due to the strong depolymerization and covalent bond breakage (Benito-Román et al., 2016). The mushroom polysaccharides *Pleurotus ostreatus* are linked in two parts, the first is on the cell surface of the mycelia, forming complexes with proteins and the second is within the cell membrane, organized into supramolecular complexes and gels networks (Tchobanian, Oosterwyck, & Fardim, 2019). Extraction of these polymers requires weakening of molecular bonds and penetration of the solvent into the cell, leading to cell disruption and subsequent solubilization and dragging of these compounds. Both water and carbon dioxide under the studied experimental conditions have important

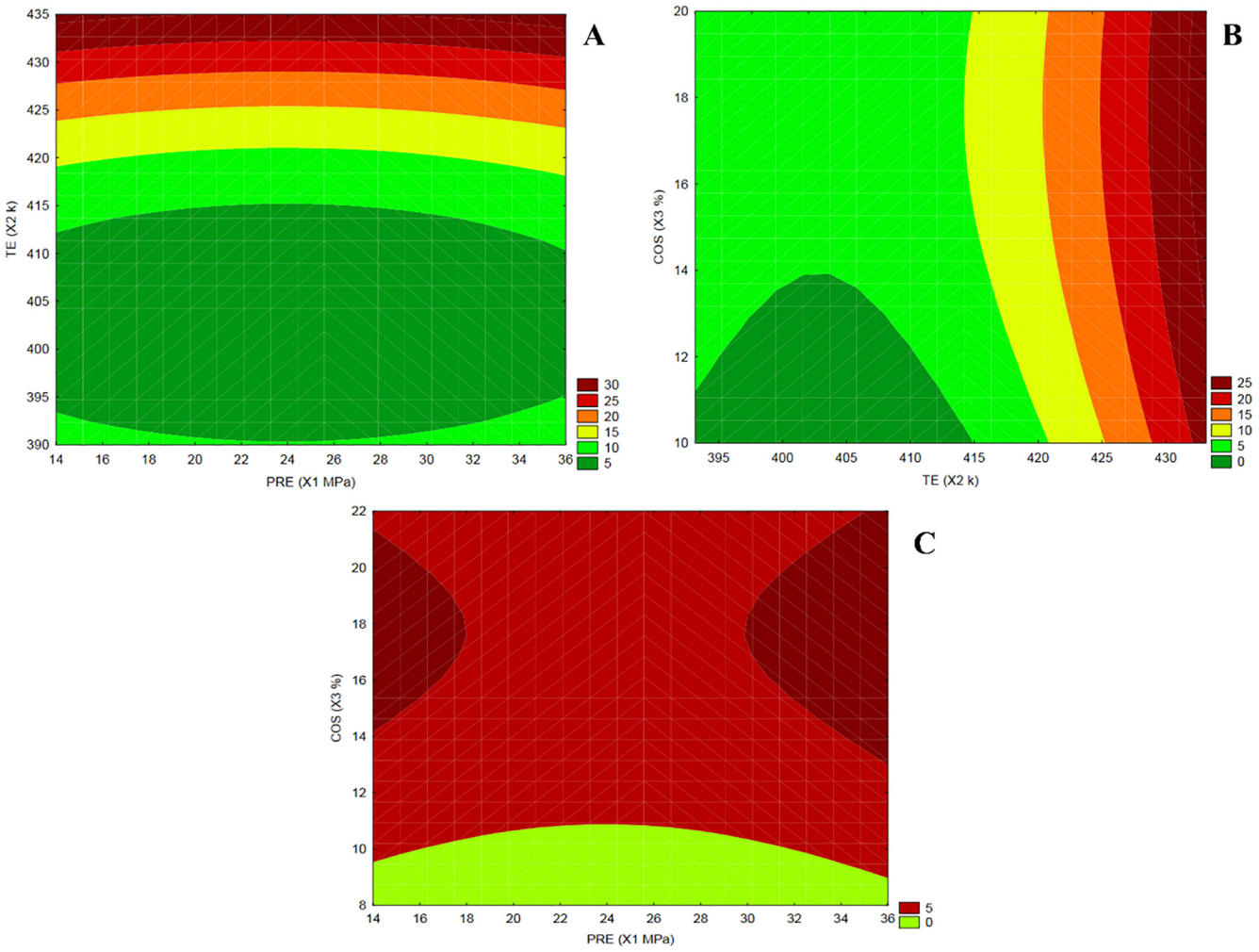

Fig. 2. Response surface level curves for overall Yield. A) Temperature × pressure; B) Cosolvent × Temperature; C) Cosolvent × Pressure.

mechanisms of action during polysaccharide extraction (Plaza & Luisa, 2019).

Hot water in the studied conditions has different and better properties compared to water at room temperature and pressure, such as low viscosity and surface tension, in addition to the reduction of the ionization self-constant (Plaza & Luisa, 2019). These properties help to weaken the bonds between polysaccharides and other molecules, improving extraction. Already supercritical CO_2 penetrates the cell wall of the fungal mycelium, swelling the cell, opening the pores and facilitating the entry of hot and pressurized water, leading to cell lysis, polysaccharide solubilization in water and its drag to the extractor outlet (Plaza & Luisa, 2019).

3.1.3. Process of obtaining polysaccharide extract in the best extraction

The trend behavior observed in (Fig. 2), indicates that experiment 12 (25 MPa, 433.15 K, and 20% H_2O) is the most suitable for the recovery of extracts rich in polysaccharides. The experiment was repeated in triplicate under the conditions evaluated yielding 30.69% of the total yield and 0.951 mg of total polysaccharide (CHO_3). The experiment was chosen due to its high global yield, polysaccharide composition, and extraction conditions. The main advantages of the H_2O + CO_2-SFE technique when compared to conventional extraction are, better yields, reduction of oxidative processes, control of process parameters and obtaining extracts without contaminants.

3.2. Characterization of polysaccharide extracts

3.2.1. Fourier transform infrared spectroscopy

Infrared spectroscopy can be applied to measure molecular vibrations, corresponding to covalent polysaccharide bonds. The infrared (IV) region, 4000–200 cm^{-1} provides accurate information about the fundamental vibrations of specific molecular groups. The infrared spectra of the crude extract, containing polysaccharide and proteins (POP-SFE-P) and the deproteinizated polysaccharide (POP-SFE-D) obtained by H_2O + CO_2-SFE are shown in (Fig. 3).

The FTIR spectrum (Fig. 3A) has wavelength regions characteristic of the presence of proteins and polysaccharides. Absorption bands near 3254 cm^{-1} correspond to amide A, OH and NH group vibrations (Jridi et al., 2014). The absorption bands near 2893 cm^{-1} correspond to amide B, group vibrations =CH and $-NH^{3+}$ (Hamzeh, Benjakul, Saeleaw, & Sinthusamran, 2018). The absorption bands at 1638 cm^{-1} are characteristics of amides I, related to the elongation vibrations of groups C=O and CN (Staroszczyk, Sztuka, Wolska, & Wojtasz-paja, 2014). The regions close to 1020 cm^{-1} correspond to COC and CO bonds of a D-glucose ring, indicate the presence of glycosidic bonds and cyclic monosaccharide structures (Hou et al., 2019).

The FTIR spectrum (Fig. 3B) has wavelength regions characteristic of the presence of polysaccharides. At 4000–3000 cm^{-1}, the spectra showed a broadband with maximum absorption (minimum transmittance) at 3340 cm^{-1} attributed to normal vibrational forms of asymmetric and symmetrical stretching of OH groups (Zhou et al., 2019). The maximum absorption peaks at 2928 cm^{-1} is similar to the beta

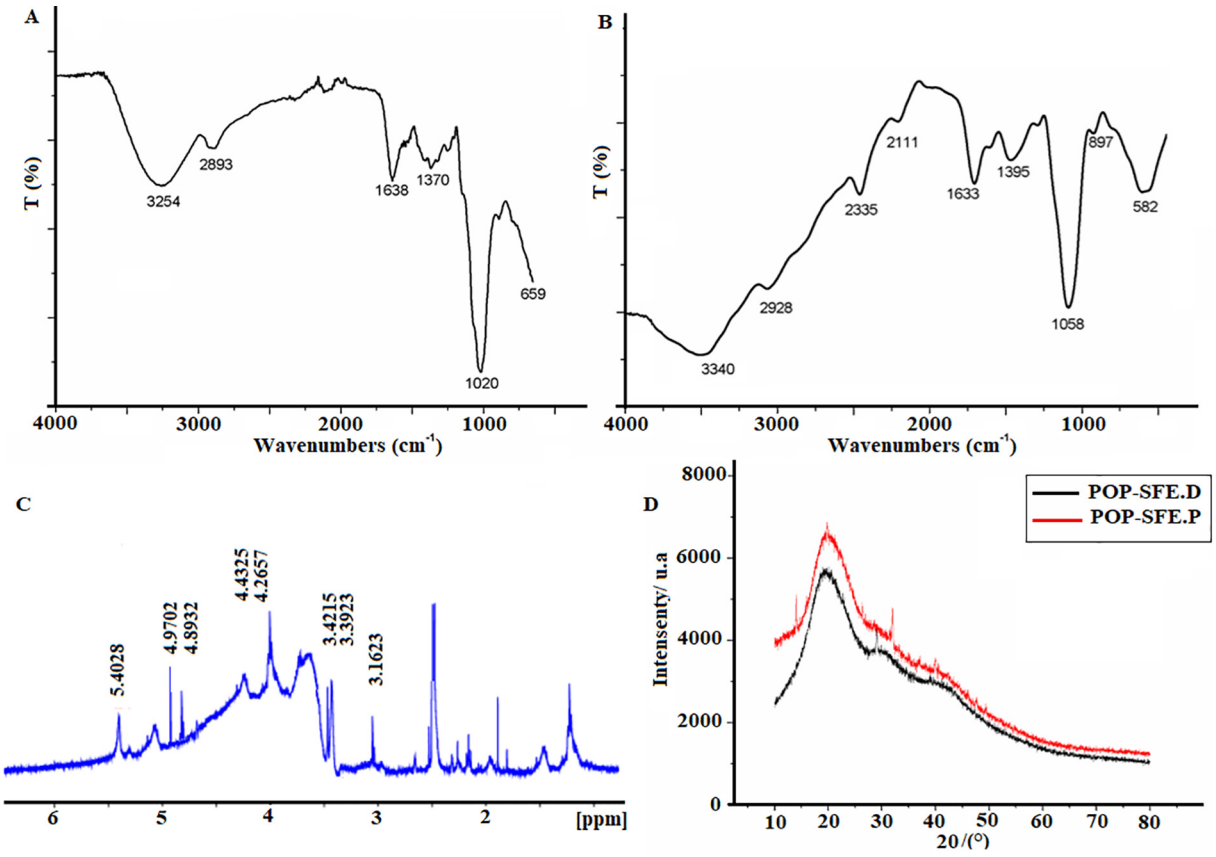

Fig. 3. Infrared spectra. A) Crude stratum, containing polysaccharides and proteins (POP-SFE-P). B) Deproteinizated polysaccharide (POP-SFE-D). C) The ^{1}H NMR spectrum (400 MHz, Me_2SO-d_6, 70 °C) of the polysaccharide extract obtained by H_2O + CO_2-SFE from the edible mushroom *Pleurotus ostreatus*. D) XRD standards measured from crude extract samples, containing polysaccharides and proteins (POP-SFE-P) and deproteinizated polysaccharide (POP-SFE-D) obtained by H_2O + CO_2-SFE.

glucan standard (Zhou et al., 2019). The maximum absorption peaks at 1633 cm^{-1} are values relative to the vibrational modes of asymmetric and symmetric CH groups (Zhou et al., 2019).

The regions 1285–1395 cm^{-1} correspond to COC and CO bonds of a D-glucose ring and to network vibrations in which all atoms of the macromolecular chain vibrate in phase and normal modes (Zhou et al., 2019). The peak regions at 1058 cm^{-1} indicate the presence of glycosidic bonds and cyclic monosaccharide structures (Hou et al., 2019). Finally, the absorption peak at 897 cm^{-1} is indicative of β-glycosidic anomeric bonds specific for β-glucans (Zhou et al., 2019). Similar FTIR results were recorded for β-glucan polysaccharides in mushrooms *Pleurotus spp*.

3.2.2. Nuclear magnetic resonance (NMR) spectroscopy

The types of anomericity and binding of the polysaccharides present in the extracts were determined by ^{1}H NMR spectroscopy (Fig. 3C). The NMR results showed POP-SFE extracts contained a mixture of polysaccharides such as heteropolysaccharides, β-glucans, α-glucans and oligosaccharides. Similar results have been observed in previous studies with *Pleurotus ostreatus* (Ruthes, Smiderle, & Iacomini, 2016; Ruthes, Smiderle, & Iacomini, 2015).

Reducing units were identified in two regions δ 4.89 and δ 4.26 (^{1}H), which indicates the presence of low molecular weight carbohydrates (Monteiro, Miguez, Pedro, & Barros, 2019). Recent reviews (Barbosa, Freitas, Martins, & Carvalho Junior, 2020), demonstrate that mushrooms of the genus *Pleurotus spp* have polysaccharides of the type β-glucans and α-glucans linked (1 → 3), (1 → 6), with various types of ramifications, monosaccharide composition and molecular weight. NMR signals show that these polysaccharide configurations were extracted from *Pleurotus ostreatus* by H_2O + CO_2-SFE. The resulting signals from δ 4.13 to δ 4.43 (^{1}H), confirm the presence of glucans C-1 with β configuration. While the signals resulting from δ 4.97 to δ 4.98 (^{1}H), confirm the presence of glucans C-1 with α configuration (Ruthes et al., 2016).

The resulting signals in the ranges of δ 3.16 to δ 3.39 (^{1}H), refer to branches at the C-3*O*-substituted of β-linkages. The resulting signal in the ranges of δ 3.55 (^{1}H), refer to branches at the C-3*O*-substituted of α-linkages (Ruthes et al., 2016). The presence of these NMR signals indicates the presence of α and β-glucans (1 → 3) substituted in the polysaccharide extracts of *Pleurotus ostreatus* obtained by H_2O + CO_2-SFE.

3.2.3. XRD standards

The X-ray diffraction patterns (DRX), crude extract, containing polysaccharide and proteins (POP-SFE-P) and deproteinized polysaccharide (POP-SFE-D) obtained by H_2O + CO_2-SFE are shown in (Fig. 3D).

The XRD patterns of the crude extract, containing polysaccharides and proteins (POP-SFE-P), show diffraction peaks at 13.2°, 20.6°, 25.6° and 32.8°. The peaks show that the material is monodisperse and homogeneous. The results are similar to other research reports with polymers (Siqueira et al., 2019). The presence of a wide 32.5° peak in the deproteinized polysaccharide diffractogram (POP-SFE-D) indicates the crystalline nature and the nanometric dimensions of the polysaccharide particles. The XDR pattern of the deproteinizated polysaccharide (POP-SFE-D) is characteristic of an amorphous and dense polymer with interpenetrating polymer chains interconnected with each other. This type of polymeric characteristic indicates the degree of disorganization of the polysaccharide structure, reveals the presence of numerous structures with different molecular weights, degrees of

branching and structural conformation (Siqueira et al., 2019).

3.3. Polysaccharide DPPH (DPPH) radical scavenging activity

The antioxidant activity of the polysaccharide extracts of *Pleurotus ostreatus* obtained by H_2O + CO_2-SFE, as well as the results of the antioxidant activity of the conventional extract, are available in the supplementary material (S4).

The results of antioxidant activity suggest that experiment 12 (25 MPa, 433.15, and 20% H_2O) has the highest antioxidant activity (80.83% ± 1.56), among the factorial design experiments. While the lowest antioxidant activity (30.25% ± 1.96) was observed in experiment 7 (15 MPa, 413.15 K, and 20% H_2O). After optimizing the extraction experiments, the best extraction condition, experiment 12, has the highest antioxidant activity when compared to all other experiments and conventional extraction (72.24% ± 2.65). The polysaccharides of *Pleurotus ostreatus* have antioxidant activity, mainly associated with the composition of monosaccharides, molecular weight, conformation of the chain, and associations of proteins (Zhang et al., 2014).

The antioxidant activity of polysaccharide extracted by H_2O + CO_2-SFE from the genus *Pleurotus spp* is similar to other mushroom polysaccharides as, *Pleurotus abalonus* (Alam, Yoon, Lee, Cho, & Lee, 2012), *Pleurotus tuber-regium* and *Pleurotus cornucopiae* (Wu, Hu, Li, Huang, & Jiang, 2014).

3.4. Polysaccharide extract (POP-SFE) has no cytotoxic effects on HepG2 cells

The cytotoxic effect of POP-SFE was evaluated *in vitro* with HepG2 cells (human hepatocarcinoma) and the results are shown in (Fig. 4). The results show that all concentrations, both at 24 and 48 h, do not show cytotoxic effects on human hepatocarcinoma cells. Due to the absence of cytotoxicity, the antioxidant activity of polysaccharide extracts was evaluated.

The cytotoxic effect on cells has been addressed in recent studies with polysaccharides (Kushairi, Phan, Sabaratnam, David, & Naidu, 2019; Morales et al., 2019), the results suggest that the mushroom polysaccharides do not show cytotoxic effects in a cell model, both *in vitro* and *in vivo*, corroborating the results presented in the present work. The cytotoxicity of polysaccharides is considered negligible due to the cellular capacity to metabolize these polymers, producing glucose monomers and other oligomers as a by-product (Morales et al., 2019). The results suggest that the consumption of mushrooms to obtain bioactive polysaccharides is safe and presents no risk of cytotoxic effects.

3.5. Polysaccharide extract (POP-SFE) protects cells from oxidative damage induced by H_2O_2

Hydrogen peroxide (H_2O_2) is generally used for studies that simulate oxidative lesions in *in vitro* systems, mainly because it is superior in oxidative action kinetics when compared with ROS (Reactive oxygen species). In addition, it has a longer half-life, which allows better oxidative activity and penetration into the cell membrane. Thus, the present study selected H_2O_2 to stimulate HepG2 cells as a model of oxidative damage (Liao & Huang, 2019). The antioxidant effects in the cell model of the polysaccharide extract of *Pleurotus ostreatus* obtained by H_2O + CO_2-SFE are shown in (Fig. 5).

The results of the MTT test (Fig. 5), indicated that the cell death rate increased significantly when the cells were exposed to 10 μM H_2O_2 for 24 and 48 h. In addition, the polysaccharide extract of *Pleurotus ostreatus* increased the rate of cell survival, indicating antioxidant activity, protecting cells from oxidative damage induced by H_2O_2 in the 48 h treatment. The concentrations of polysaccharide extract when compared with the peroxide control do not differ significantly ($p < 0.05$) in relation to antioxidant activity. However, in relation to the exposure time, it is observed that in 48 h, the viability of cell protection is greater when compared in 24 h. In addition, even the lowest concentration of POP-SFE has antioxidant activity.

Oxidative stress is an imbalance between the production of free radicals and the cellular ability to neutralize the side effects of the imbalance with antioxidant agents. High concentrations of free radicals and reactive species are harmful to the living organism, damaging the cell membrane and DNA, leading to inflammatory processes and cell death. In response to the accumulation of free radicals, defense cells such as macrophages are activated. However, in some cases the excessive production of free radicals unbalances the natural defense mechanisms and triggers mutagenic processes in the cells themselves (Liao & Huang, 2019). Mushroom polysaccharides have antioxidant activity as shown in recent reports (Li et al., 2019; Liao & Huang, 2019). The main mechanisms can be associated with an indirect action, so the polysaccharides can bind to specific receptors such as Toll-like receptor 4 (TLR4), complement receptor 3 (CR3), sequester receptor, dectin-1

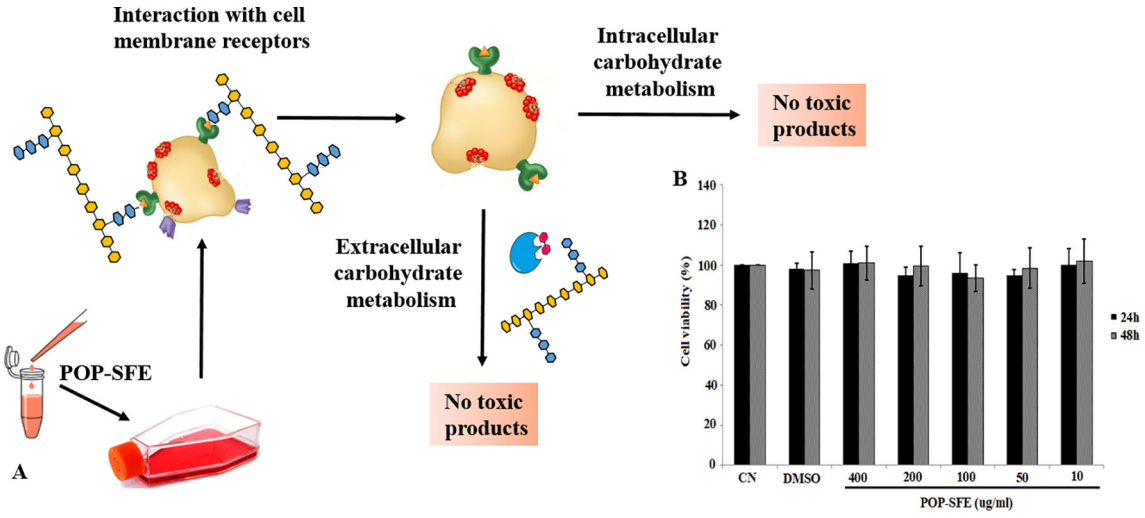

Fig. 4. Graphic summary. A) Schematic representation of the cycle of biochemical mechanisms that lead to the consumption and degradation of carbohydrate polymers. Polysaccharides bind to cell membrane receptors inducing the cell to two cycles of degradation (intracellular and extracellular), both of which do not produce toxic by-products. B) Cytotoxicity of polysaccharide extracts (POP-SFE). The results were expressed as percentage of viable cell versus negative control. All values presented correspond to the mean ± SD.

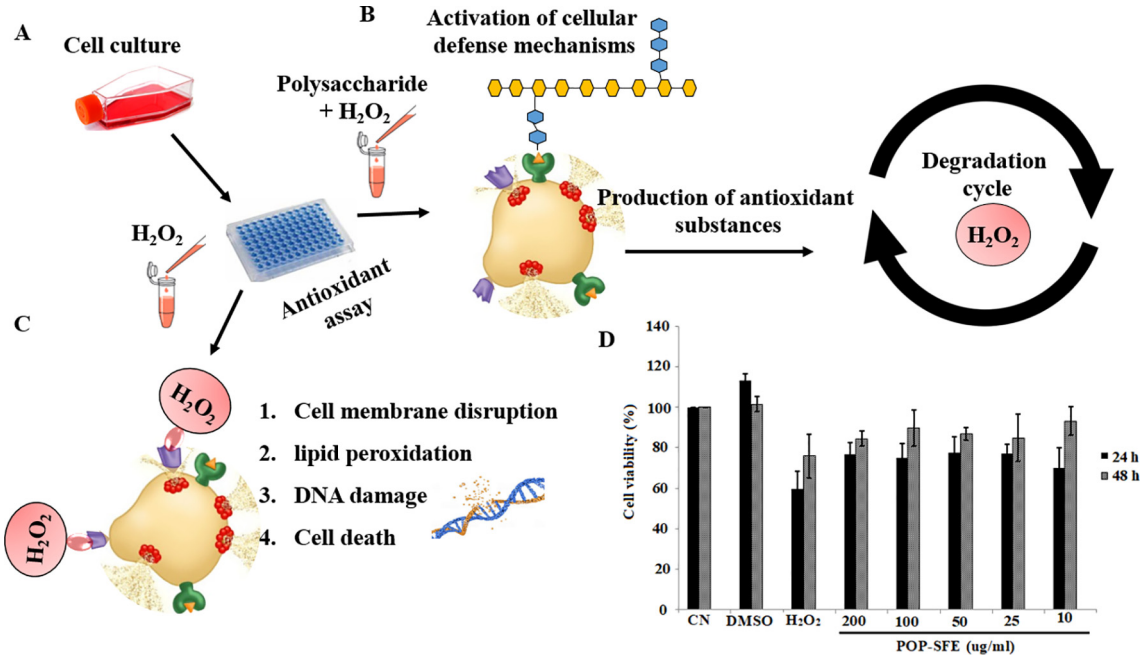

Fig. 5. Graphic summary. A) Cell culture and antioxidant activity assay. B) Representation of the action of polysaccharides linked to cellular receptors. Pattern recognition through cell wall receptors leads the cell to produce various antioxidant substances, initiating a degradation cycle of hydrogen peroxide. C) Schematic representation of the hydrogen peroxide action in unprotected cells (without the presence of the polysaccharide), leading to a state of cell membrane degradation, lipid peroxidation and DNA damage. D) Antioxidant effects of POP-SFE in the treatment of HepG2 cells against oxidative damage induced by H_2O_2. All values presented correspond to the mean ± SD of three independent experiments (n = 3).

and receptor mannose, activating the adaptive defense system (Barbosa et al., 2020).

The activation of the defense system by the synergistic action of the polysaccharides with the cell membrane receptors, triggers a cycle of specific biochemical phenomena, leading to the production of antioxidant and anti-inflammatory substances such as of TNF-α and IFN-γ. The production of these substances is controlled by several genes coupled to a network of biochemical phenomena, which is kinetically longer, which justifies the best cytoprotective activity in 48 h. In addition, the conformation and distribution of the polysaccharide along the cell membrane can provide a steric impediment to hydrogen peroxide molecules, limiting their direct action against the cell membrane in the first 24 h (Barbosa et al., 2020).

Studies such as that by Song, Ren, and Zhang (2019) showed that mushroom polysaccharides can improve the antioxidant capacity of intracellular substances, regulating the intracellular production of antioxidants and enzymes that inhibit the action of peroxide hydrogen. Polysaccharides can induce cells to an antioxidant activity cycle, inhibiting ROS production, regulating the cell cycle, preventing damage to DNA and protecting cell membrane (Song et al., 2019).

4. Conclusions

Binary hot water technology with the addition of supercritical carbon dioxide (H_2O + CO_2-SFE) was used successfully to obtain extracts rich in antioxidant polysaccharides from the edible mushroom *Pleurotus ostreatus*. The application of the Box-Behnken statistical project helped to determine the best extraction conditions. After defining the extraction conditions, high extraction yields and polysaccharide composition were obtained. Chemical analysis shows that polysaccharide extracts are a mixture of heteropolysaccharides, β-glucans, α-glucans, and oligosaccharides. All extracts obtained have antioxidant activity by the DPPH model. However, the extraction condition defined by the desirability function showed the highest antioxidant activity and no cytotoxic effect in an *in vitro* cell model. In addition, the extract showed antioxidant activity in the cell model, protecting cells against oxidative damage induced by H_2O_2. The extraction technology (H_2O + CO_2-SFE) proved to be efficient, innovative, and ecological to obtain high yields of polysaccharide extracts.

CRediT authorship contribution statement

Jhonatas Rodrigues Barbosa: Conceptualization, Investigation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Maurício Madson S. Freitas:** Methodology, Data curation, Writing - review & editing. **Luà Caldas Oliveira:** Methodology, Data curation. **Rafael Maia Oliveira:** Methodology, Data curation. **Julio Cesar Pieczarka:** Writing - review & editing. **Davi do Socorro B. Brasil:** Writing - review & editing. **Raul Nunes Carvalho Junior:** Writing - review & editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.127173.

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CONCLUSÃO GERAL

Após extensa revisão da literatura ficou claro que os polissacarídeos de cogumelos do gênero *Pleurotus spp* possuem propriedades fascinantes. Foi demostrado que estes polímeros naturais possuem propriedades incríveis, são biodegradáveis, biocompatíveis e não são tóxicos. Além disso, podem ser extraídos usando técnicas inovadoras de extração como a tecnologia de fluido supercrítico. As bioatividades destes polímeros foram demonstradas e seus mecanismos elucidados. Os achados da literatura demostram que os polissacarídeos possuem habilidade química e molecular para se ligar de forma eficiente a receptores celulares, sendo uteis para diversa aplicações. Com base nestas propriedades novas tecnologias como selenização e produção de vacinas usando como plataforma os polissacarídeos tem sido desenvolvidas com sucesso. Estas novas tecnologias mostram que os polissacarídeos são produtos de interesse comercial e industrial.

A pesquisa usando um sistema binário de água quente e CO_2 supercrítico foi eficiente para recuperação de extratos ricos em polissacarídeos do cogumelo *Pleurotus ostreatus*. Após a otimização do leito e das condições de extração foram obtidos extratos ricos em polissacarídeos de forma eficiente, com menor tempo e usando solventes verdes. Os extratos caracterizados demostram ter uma composição rica em vários polissacarídeos como α e β-glucanos, além de heteropolissacarídeos e outros de baixo peso molecular. Foi confirmado a que os extratos possuem atividade antioxidante em modelos como DPPH. Além disso, estudos *in vitro* usando células HepG-2, mostram que o extrato da melhor condição de extração não apresenta efeitos citotóxicos, mais apresenta atividade citoprotetora contra danos oxidativos induzidos por H_2O_2. Por fim, a tecnologia e o processo desenvolvidos foram eficientes para a recuperação de extratos ricos em polissacarídeos com atividade antioxidante e citoprotetora. Além disso, a tecnologia no futuro pode ser parametrizada para produção destes polímeros em larga escala.

APÊNDICE

Publicações como Primeiro autor

Apêndice I. Artigo publicado na Carbohydrate Polymers 2020: Occurrence and possible roles of polysaccharides in fungi and their influence on the development of new technologies.

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Review

Occurrence and possible roles of polysaccharides in fungi and their influence on the development of new technologies

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ABSTRACT

The article summarizes the roles of polysaccharides in the biology of fungi and their relationship in the development of new technologies. The comparative approach between the evolution of fungi and the chemistry of glycobiology elucidated relevant aspects about the role of polysaccharides in fungi. Also, based on the knowledge of fungal glycobiology, it was possible to address the development of new technologies, such as the production of new anti-tumor drugs, vaccines, biomaterials, and applications in the field of robotics. We conclude that polysaccharides activate pathways of apoptosis, secretion of pro-inflammatory substances, and macrophage,

Apêndice II. Capitulo de livro publicado na Springer Nature Switzerland AG 2019: Application of Mycogenic Nanoparticles Against Neurodegenerative Diseases.

Chapter 5
Application of Mycogenic Nanoparticles Against Neurodegenerative Diseases

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Abstract Metallic nanoparticles biosynthesis studies such as gold, silver, selenium, iron, metal oxides, and others, by microorganisms, especially fungi, have received great interest from the international scientific community because it is an ecological alternative. This chapter aims to evaluate recent advances in biosynthesis of fungal nanoparticles, with emphasis on physicochemical properties, bioactivity, and applications. Recent advances in biotechnology have provided interesting tools for the controlled synthesis of nanoparticles, using fungi as biotechnological factories. Due to the great diversity of polysaccharide enzymes and polysaccharide structures synthesized by fungi, it makes this kingdom very promising for the nanoparticles biosynthesis. The synthesized nanoparticles present different physicochemical properties, as they depend on biochemical parameters related to the biosynthesis process, besides the conditions used during the process, such as temperature, pH and substrate concentration. Metal nanoparticles associated with

Apêndice III. Capitulo de livro aceito para publicação na Springer Nature, 2020. Polysaccharides extraction and processing using green solvents: recent developments, challenges, and new opportunities

Polysaccharides extraction and processing using green solvents: recent developments, challenges, and new opportunities

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Apêndice IV. Capitulo de livro aceito para publicação na Materials Research Forum, USA, 2020. Polymer aerogels: Preparation and potential for biomedical application.

Polymer aerogels: Preparation and potential for biomedical application

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Apêndice V. Capitulo de livro aceito para publicação na Materials Research Forum, USA, 2020. Bioaerogels: Synthesis Approaches, Biomedical Applications and Cell Uptake.

Bioaerogels: Synthesis Approaches, Biomedical Applications and Cell Uptake

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Apêndice VI. Capitulo de livro aceito para publicação na Wiley-Scrivener Publishing, 2020. Application of polysaccharides in cancer treatment.

Application of polysaccharides in cancer treatment

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Wiley-Scrivener Publishing.

Apêndice VII. Capitulo de livro aceito para publicação na Wiley-Scrivener Publishing, 2020. Polysaccharides-based metal-organic frameworks.

Polysaccharides-based metal-organic frameworks

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Apêndice VIII. Capitulo de livro aceito para publicação na Elsevier, 2020. Influence of solid state fermentation, submerged fermentation and Biphasic fermentation on the production of biosurfacatan.

Influence of solid state fermentation, submerged fermentation and Biphasic fermentation on the production of biosurfacatan

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Apêndice IX. Outras contribuições cientificas na forma de artigos e capítulos de livros.

1. **Artigo publicado:** De Oliveira, Luã Caldas; Rodrigues Barbosa, Jhonatas; Da Conceição Amaral Ribeiro, Suezilde; De Vasconcelos, Marcus Arthur Marçal; De Aguiar, Bruna Araújo; Da Silva Pereira, Gleice Vasconcelos; Albuquerque, Gilciane Américo; Da Silva, Fabricio Nilo Lima; Lopes Crizel, Rosane; Campelo, Pedro Henrique; De Fátima Henriques Lourenço, Lúcia. Improvement of the characteristics of fish gelatin-gum arabic through the formation of the polyelectrolyte complex. Carbohydrate Polymers, v. 233, p. 115068, 2019.
2. **Artigo submetido e aceito:** Geographical discrimination of Amazonian cacao by near infrared spectroscopy: influence of sample preparation. Computers and Electronics in Agriculture, 2020.
3. **Artigo submetido:** Application of the near infrared spectroscopy technique to evaluate quality and compositional atributes of cocoa: an overview. Food Chemistry, 2020.
4. **Artigo submetido:** Polysaccharide extract of mushroom Pleurotus ostreatus improves properties technological features of fish gelatin film, Carbohydrate Polymers, 2020.
5. **Capitulo de livro publicado:** Santos, Janaina Pompeu dos; Silva, Sabrina Baleixo da; Santos, Renato Meireles dos; Barbosa, Jhonatas Rodrigues; Aires, Cassia Barbosa; Portilho, Martina Damasceno; Batista, Flaviane Leal; Freitas, Joice Silva de; Silva, Lucas Henrique da Silva e; Silva, Natacia da Silva e; Araújo, Wanessa Shuelen Costa; Dantas, Vanderson Vasconceslos. Avaliação das características físicoquímicas de sementes de andiroba (carapa guianensis - meliaceae) e açaí (euterpe oleracea). In: Edson da Silva. (Org.). Tópicos Multidisciplinares em Ciências Biológicas. 1ed.Ponta Grossa, PR: Atena Editora, 2020, v. 1, p. 9-15.
6. **Capitulo de livro publicado:** Menezes, Eduardo Gama Ortiz; Barbosa, Jhonatas Rodrigues; Siqueira, Leticia Maria Martins; Carvalho Junior, Raul Nunes de. Modelagem termodinâmica e determinação da solubilidade do óleo de bacaba (oenocarpus bacaba) e ucuúba (virola surinamensis) com dióxido de carbono supercrítico. In: Vanessa Bordin Viera, Natiéli Piovesan. (Org.).

Inovação em Ciência e Tecnologia de Alimentos 2. 1ed.Ponta Grossa, PR: Atena Editora, 2019, v. 2, p. 228-236.

7. **Capitulo de livro publicado:** Paula de Souza e Silva, Ana; Almeida da Costa, Wanessa; de Los Angeles Rodriguez Salazar, Marielba; do Nascimento Bezerra, Priscila; Cristina Seabra Pires, Flávia; Caroline Rodrigues Ferreira, Maria; Gama Ortiz Menezes, Eduardo; Rafael Olivo Urbina, Glides; Rodrigues Barbosa, Jhonatas; Carvalho Raul Nunes, de. Commercial and Therapeutic Potential of Plant-Based Fatty Acids. Biochemistry and Health Benefits of Fatty Acids. 1ed.London: IntechOpen, 2018, v., p. 1-17.
8. **Capitulo de livro submetido e aceito:** Properties of polysaccharides, Wiley-Scrivener Publishing, 2020.
9. **Capitulo de livro submetido e aceito:** Extraction of Antioxidants of Vegetable Matrices with Green Solvents. Springer, 2020.
10. **Capitulo de livro submetido e aceito:** Supercritical Green Solvent for Amazonian Natural Resources. Springer, 2020.
11. **Capitulo de livro submetido e aceito:** Aerogels envisioning future applications. Materials Research Forum, USA, 2020.
12. **Capitulo de livro submetido e aceito:** Traditional uses, phytochemicals and pharmacological properties of the plant Chenopodium ambrosioides. Elsevier, 2020.