Carbohydrate Polymers 117 (2015) 753–761

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Review

D-Glucans from edible mushrooms: A review on the extraction, purification and chemical characterization approaches

Andrea Caroline Ruthes, Fhernanda Ribeiro Smiderle, Marcello Iacomini*

Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, Centro Politécnico, Curitiba CEP 81531-980, Brasil

ARTICLE INFO

Article history:
Received 13 August 2014
Received in revised form
29 September 2014
Accepted 16 October 2014
Available online 30 October 2014

Keywords:
D-Glucans
Edible mushrooms
Extraction
Purification
Chemical characterization

ABSTRACT

D-Glucans from edible mushrooms present diversified chemical structures. The most common type consists of a backbone of β-D-glucose (1 → 3)-linked frequently branched at O-6 by β-D-glucose residues as side chains. However it is possible to distinguish α-, β- and mixed D-glucans. Further discrimination could be made on the basis of glycosidic bond position in a pyranoid ring, distribution of specific glycosidic bonds along the chain, branching and molecular weight. The present manuscript reviews the processes of extraction, purification and chemical characterization of D-glucans, such as NMR studies, methylation analysis, Smith degradation, and some other methodologies employed in carbohydrate chemistry characterization. In addition, these polysaccharides are important because they can provide many therapeutic benefits related to their biological activity in animals and humans, either immunostimulatory activity, inhibiting tumor growth, as well as exerting antinociceptive and anti-inflammatory action, among others, which are usually attached to their structure, molecular weight and degree of branching.

Contents

* Corresponding author. Tel.: +55 41 3361 1655; fax: +55 41 3266 2042.
E-mail address: iacomini@ufpr.br (M. Iacomini).

http://dx.doi.org/10.1016/j.carbpol.2014.10.051
0144-8617/

1. Introduction to mushroom D-glucans

Mushrooms have been appreciated and consumed for their nutritional value and medicinal properties in oriental countries for more than 2000 years. These fungi have traditionally been used for the prevention and also treatment of a multitude of disorders, and they have been increasingly consumed by cancer patients, during their treatments, as dietary supplements (Moradali, Mostafavi, Ghods, & Hedjaroude, 2007; Hardy, 2008). Researchers have considered these fungi as healthy food because they are good sources of vitamins, minerals, proteins, and carbohydrates, apart from low level of lipids and low caloric content (Wasser, 2002; Kalac, 2009). Fungi are excellent sources of D-glucans. They can present α-D-glucans, β-D-glucans, and also α/β-D-glucans in their fruit bodies (Synytsya & Novák, 2013). Mostly of these homopolysaccharides are not digested by human enzymes, being considered as fibers, which can assist intestinal motility and increase stool bulk, decreasing absorption of harmful toxic and carcinogenic substances, leading to lower incidence of cancer (Cheung, 2013).

Despite of the fact that mushroom D-glucans have presented to possess a variety of therapeutic benefits, the study on their chemical structures still requires more attention, especially in the fields of purification and chemical characterization. It was observed that the differences among the D-glucan structures may affect their biological properties (Giavasis, 2014). The glucans isolated from basidiomycetes present different linkage types, branching degrees, molecular weight, and solubility, therefore their chemical structures should be carefully determined with the aim of finding the best chemical structure for the desired therapeutics.

There are a plenty of methods to extract, purify and characterize these polysaccharides. This review attempts to summarize the methods for obtaining purified or crude D-glucans, as well as the techniques used for their chemical characterization and also to revise the structures of the D-glucans isolated from edible mushrooms up to this date. For this purpose, a search was performed using the Science Direct database, using the keywords combination: basidiomycete + homopolysaccharide + glucans, from 2004 to 2014. The most relevant findings were reported herein, as well as some classical articles about chemical reactions still used in carbohydrate analysis.

2. D-Glucans conventional extraction methods

The identification and characterization of carbohydrate structures requires, the isolation and purification of the polymer of interest from its original source. Regarding mushroom polysaccharides, they could be obtained from fruit bodies, mycelial biomass or directly from the liquid culture broth, as released exopolysaccharides (EPS). Besides that, spores and sclerotium from mushrooms also could be considered as sources for the extraction of glucans (Dong et al., 2012; Wang et al., 2014).

In general, D-glucans are commonly extracted using water in different temperatures or alkaline aqueous solutions. Therefore, these types of extraction will be covered in sequence.

2.1. Aqueous extraction

Powdered fruit bodies can be firstly extracted with some organic solvent, generally EtOH, acetone, or mixtures of $CHCl_3$:MeOH, to remove apolar compounds such as lipids, phenols and terpenes, although this is not demanded. The organic solvent extraction facilitates the complete separation of polysaccharides from other compounds that may be present (Carbonero et al., 2006; Dore et al., 2007; Smiderle et al., 2008a; Bi et al., 2009; Synytsya et al., 2009; Wang, Yu, & Mao, 2009; Klaus et al., 2011). The polysaccharide extraction is, traditionally, carried out with water at room temperature (Santos-Neves et al., 2008a,b; Smiderle et al., 2008a; Palacios, Garcia-Lafuente, Guillamón, & Villares, 2012; Smiderle et al., 2013). Depending on the aim of the study, this procedure could be repeated to exhaustion, in order to do a previous step of polysaccharide fractionation. After centrifugation, the supernatant, named cold water extract is separated from the residue. Therefore, the residue can be extracted with boiling water and again separated from the hot water extract by centrifugation (Santos-Neves et al., 2008a,b; Smiderle et al., 2008a; Palacios et al., 2012; Smiderle et al., 2013).

The exhaustive repetition of the extraction procedure contributes to enhance the yield of polysaccharide fractions, besides it diminishes the mixture of polysaccharides regarding the different extracts.

More advanced technologies to obtain polysaccharides have been used recently, as ultrasonic (Chen, Wang, Zhang, & Huang, 2012) and microwave assisted extractions (Zeng, Zhang, Gao, Jia, & Chen, 2012), besides the pressurized solvent extraction (Palanisamy et al., 2014). The latter procedure showed to be faster and more efficient in obtaining higher yield of polysaccharides, comparing to the traditional methodologies. Although it requires more specific devices, which increases the costs (Palanisamy et al., 2014).

2.2. Alkaline extraction

The remainder residue from aqueous extraction may be subsequently extracted with aqueous basic solutions (NaOH or KOH, 2% w/v) at 100 °C, but these conditions could vary (Smiderle et al., 2006; Amaral et al., 2008; Santos-Neves et al., 2008b; Synytsya et al., 2009; Chen, Zhang, & Cheung, 2010; Palacios et al., 2012; Song & Du, 2012; Ruthes et al., 2013a; Maity et al., 2014). Again, the residue is separated by centrifugation and the supernatant give rise to the alkaline extract. Extraction with basic aqueous solutions frequently is done using $NaBH_4$ to protect reducing end-units avoiding degradation of polysaccharide chains (Smiderle et al., 2006; Santos-Neves et al., 2008b).

3. Purification methods

After polysaccharide extraction, samples could be submitted to several purification steps to remove other substances such as proteins, phenolic compounds, monossacharides, amino acids or other related molecules. Proteins can be removed by precipitation with trichloroacetic acid (20%, w/v), by treatment with the enzyme protease at 40 °C for 1 h (pH 7.5), using Sevag method (Staub, 1965), or by treatment with phenolic reagent (Synytsya et al., 2009).

Moreover, several purification steps must be carried out in order to obtain pure polysaccharide fractions. The most common purification process used to obtain pure D-glucan fractions are listed below.

3.1. Freeze–thawing

Freeze–thawing is a very simple and efficient process in order to obtain pure D-glucans.

In this process the crude extract in concentrated aqueous solution is frozen and then slowly thawed at room temperature, for several times (Gorin & Iacomini, 1984). Following centrifugation of the precipitate, both supernatant and precipitate should be once again frozen and then slowly thawed till there is no formation of precipitate from the supernatant fraction. D-Glucans are generally found at precipitate fraction.

The principle of this method is based on differences among polysaccharide branch degrees. Those molecules presenting a linear structure or fewer branches tend to precipitate, while those with a higher branching degree remain in the cold-water soluble fraction (Ruthes et al., 2013a).

3.2. Treatment with solvents

The D-glucans are able to form complexes (by hydrogen bonds) with other polysaccharides and also other classes of molecules (phenols, terpenes, proteins). As a result, the D-glucan extracts obtained are not pure, retaining these compounds, which can interfere the chemical analysis and also their bioactivity. This may cause a misinterpretation of the results, leading to erroneous conclusions. The most common solvent treatment is the precipitation with 2 or 3 volumes of cold ethanol (Smiderle et al., 2006; Ruthes et al., 2013a; Dong et al., 2012). This solvent dehydrate the polysaccharides and make them precipitate, separating these high molecular weight molecules from the low molecular weight ones. Besides, the D-glucans in β-configuration are usually more soluble in apolar solvents as dimethyl sulfoxide. Therefore, the treatment with this solvent is an effective method to separate β-D-glucans from other polysaccharides or water-soluble D-glucans (Smiderle et al., 2013).

The treatment with NaOH solution, in different concentrations, can also result in separation of D-glucans from other compounds because this solution interferes in the helical 3-D conformations of the D-glucans, leading to a better separation of contaminants (Smiderle et al., 2008a; Lehtovaara & Gu, 2011).

3.3. Treatment with Fehling solution

Copper salts are widely used to fractionate mixtures of polysaccharides (Jones & Stoodley, 1965). Fehling solution is often employed because it contains Cu(II), metal ions, which permits the formation of complexes at the presence of reactive groups (COOH, OH, NH_2).

The treatment with Fehling solution is not very common regarding to D-glucans purification, but in cases that such homopolymers remain in cold-water soluble fraction after freeze thawing, mixed with heteropolysaccharides, it becomes an effective process (Ruthes et al., 2013b).

In general, cold-water soluble fractions after freeze thawing are submitted to treatment with Fehling solution. The Fehling solution consists of a combination of two solutions, named A and B, that are mixed in equal volumes (Jones & Stoodley, 1965). Over the years this solution was modified, the A solution is composed of Copper(II) sulfate ($CuSO_4{\cdot}5H_2O$; 55.7 g; H_2O q.s.p. 500 mL). While, solution B is composed of potassium sodium tartrate tetrahydrate ($KNaC_4H_4O_6{\cdot}4H_2O$; 173 g) plus potassium hydroxide (KOH; 125 g) in aqueous solution (q.s.p. 500 mL).

Polysaccharide fractions are first solubilized in solution B, and the same volume of solution A is added. The volume of solution B used to solubilize the sample depends on the fraction, and only the minimum volume necessary to solubilize different polysaccharide fractions may be used in order to optimize the fractionation.

After addition of Fehling solution, the mixture is maintained under magnetic stirring at room temperature (~12 h), and subsequently the material should be kept at 4 °C overnight. Soluble and insoluble-copper complexes must be separated by centrifugation, neutralized and dialyzed against tap water (~48 h). After that, both fractions must be deionized with mixed ion exchange resins, once again dialyzed against tap water (~24 h) and then freeze dried.

3.4. Closed dialysis and ultrafiltration

As well as treatment with Fehling solution, the techniques of closed dialysis and ultrafiltration are not very common applied to D-glucan purifications. However, few researchers use these methods and they should be more explored since the yield loss of the samples is thereby reduced.

Fractions should be solubilized in aqueous solution at a concentration of 10 mg mL^{-1}. There are different types of membranes, as regenerated cellulose, which have been found extensive commercial applications, and polyethersulfone, as well as different MWCO, which is selected based on samples refractive index elution profiles (5–1000 kDa; Sepctra/Por®; Milipore) (Zhang, Zhou, Yang, & Chen, 1998).

Dialyses are done in closed systems, changing the elution water till negative result to total carbohydrate test (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), while ultrafiltration occurs using adequate filtration systems (*e.g.* Sartorius model 16249) coupled to a compressed air cylinder.

Although these methodologies present themselves as a highly effective method in purifying polysaccharide fractions (Amaral et al., 2008; Carbonero et al., 2008; Santos-Neves et al., 2008a; Smiderle, Carbonero, Sassaki, Gorin, & Iacomini, 2008b; Smiderle et al., 2008c; Komura et al., 2010; Ruthes, Rattmann, Carbonero, Gorin, & Iacomini, 2012; Ruthes et al., 2013a,c), with little or no loss of sample yield, it is only possible to establish a pattern of polysaccharide purification in practice.

There is great difficulty in establishing which MWCO membrane must be used for each polysaccharide mixture, since the pore size of the membranes are designed for protein purification. Therefore the polysaccharides do not follow the same rule of elution of proteins, which means that, even showing higher M_w, polysaccharides can be retained by or be eluted through dialysis or ultrafiltration membrane with a given MWCO. This occurs because these molecules may acquire different conformations in solutions, and not only their Mw, but also their behavior and conformation in aqueous solution may influence the elution profile and the purification process using dialysis or ultrafiltration.

3.5. Column fractionation

Other way to obtain pure polysaccharide fractions is the column fractionation. Crude extracts may contain several polysaccharides with different molecular sizes. Size exclusion chromatography (SEC) is the most common method used because it allows the separation of polysaccharides according to their size, and also permits the subsequent determination of their molecular weight.

Polysaccharide fractionation using SEC is becoming more important in both industrial and laboratory processes (Zhang et al., 1998). The column packing materials have been made of cross-linked dextran, agarose and polyacrylamide.

Although the SEC column is the most appropriate to separate neutral polysaccharides, a DEAE-cellulose column was used successfully to separate an α-D-glucan, that did not bind to the column and it was eluted with water (Smiderle et al., 2010). Therefore, ion

exchange chromatography can also be used, especially to separate neutral polysaccharides from charged ones.

4. Available techniques for structure elucidation

Polysaccharides are challenging to analyze due to their high diversity of composition, linkages, configuration, ring conformation, and molecular weight. In addition, many carbohydrate characterization techniques are difficult, costly and time-consuming, which requires specialized chemical and biochemical training and the development of new tools and methodologies. The characterization of glycans is driven primarily by the application of chemistry-related techniques, and the tools commonly used to characterize these polymers include gas and/or liquid chromatography, mass spectrometry, nuclear magnetic resonance (NMR), and thin layer chromatography (TLC).

4.1. Monosaccharide composition

The polysaccharides can be composed by different monosaccharides assuming pyranosidic or furanosidic conformations. In the case of homopolysaccharides, such as D-glucans isolated from mushrooms, the glucose is the only component. There are different ways to analyze the monosaccharide composition of these polymers, such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC).

The most popular use of TLC of carbohydrates is performed in Silica Gel G-60 plates, followed by detection of the carbohydrates with Orcinol-H_2SO_4 spray, since it is highly sensitive to sugars and each monosaccharide reacts differently showing a specific color. However, depending on the sugar concentrations, the color intensities may vary, and some color variations may appear (Sassaki, de Souza, Cipriani, & Iacomini, 2008a).

Monosaccharide composition can also be determined using HPLC, by their elution profile on the column, or GC. The latter technique requires derivatization of the carbohydrates, as silylation, fluoroacylation, or acetylation. Acetates have been widely employed, although silylation of carbohydrates were the simplest method of derivatization, being rapid and applicable to all carbohydrate classes (monosaccharides, alditols, uronic acids, deoxy, amino sugars, and oligosaccharides). Any of these reactions generate many isomers from each monosaccharide; therefore a previous derivatization step is necessary (reduction with sodium borohydride), such as conversion to alditols or dithiocetals (Sassaki et al., 2008b). It is not common to observe uronic acids or amino sugars on D-glucans isolated from mushrooms; therefore the simple technique of hydrolysis, reduction with sodium borohydride, followed by acetylation is effective to determine its monosaccharide profile by GC. Besides, Sassaki et al. (2008b) described a simple modification step for acetylation, in order to analyze neutral, amino and acidic monosaccharides as their alditol acetates. This approach requires small amounts of sample (1 mg) and solvents, being an effective, rapid and economic method.

4.2. Molecular weight

It has been shown that differences in solubility in water, molecular weight, branching ratio, chemical structure, and chain conformation may affect the bioactivity of D-glucans (Zhang, Li, Wang, Zhang, & Cheung, 2011; Bae et al., 2013). This justifies the necessity of evaluating the molecular weight of the isolated D-glucans. The common used techniques are high performance size exclusion chromatography (HPSEC) (Carbonero et al., 2012; Ruthes et al., 2013a; Liu et al., 2014a), gel-permeation chromatography (GPC) (Nandi et al., 2014), and high performance gel-permeation chromatography (HPGPC) (Song & Du, 2012). These techniques can be coupled to refractive index detectors (RI) and laser light scattering detector (LLS), which provide precise information on the molecular weight of the polysaccharide without the necessity of using standard samples. Although this technique is more reliable, there are many scientists that use approaches with dextran standards, as T-200, T-70, and T-40, etc. (Mandal et al., 2010; Song & Du, 2012). Unfortunately, the molecular weight is not provided for all the D-glucans isolated from mushrooms. The poor solubility of some D-glucans, mainly the β-D-glucans, may be an impediment for the success of this analysis.

4.3. Methylation analysis

The methylation analysis is the most common technique to determine the linkage type of polysaccharides. This derivatization is based on the method described by Ciucanu and Kerek (1984), with modifications (Smiderle et al., 2008a; Ruthes et al., 2010). The samples (10 mg) are dissolved in dimethyl sulfoxide (1 mL), and powdered NaOH (20 mg) plus iodomethane (CH_3I) (1 mL) are added. After 30 min of vigorous stirring, at room temperature (~25 °C), the tube reaction is maintained on the bench overnight. The reaction can be interrupted by addition of water, neutralized with HOAc, dialyzed against distilled water and freeze-dried. After this, the products can be submitted to one more cycle of methylation, and then partitioned between chloroform ($CHCl_3$) and water, and the lower chloroform layer evaporated to dryness. The resulting per-*O*-methylated products (1–2 mg) can be directly submitted to acid hydrolysis.

For insoluble D-glucans, another treatment can be used previous the methylation, to guarantee the complete methylation of the free-hydroxyls; this procedure was developed by Haworth (1915), and uses 40% aq. NaOH and dimethyl sulfate (Santos-Neves et al., 2008b). For this procedure, first the isolated polysaccharide (10 mg) should be reduced in a solution of sodium borohydride (pH 9,0), to prevent partial degradation, followed by dialysis against tap water and freeze-drying. Then, the sample is solubilized in 40% aq NaOH (w/v) (3 mL) and Me_2SO_4 (3 mL) is added dropwise (5 aliquots of 0.5 mL). The sample is maintained under continuous stirring during the entire process and for the subsequent 12 h. This process, can be repeated by adding 40% aq NaOH (w/v) (3 mL) and Me_2SO_4 (3 mL), at the same conditions. After the 12 h period of reaction of the second cycle, the sample is neutralized with HOAc, dialyzed against tap water (~24 h) and freeze-dried. Complete per-*O*-methylation of the polysaccharide is carried out using NaOH–Me_2SO–CH_3I as previously described.

After methylation, the same acetate derivatization is necessary to load the samples on GC. The inclusion of mass-spectrometry (MS) detectors coupled to GC was the greatest advance, showing highly resolved chromatograms that allow identification and quantification of compounds, even when present in the range of picograms or less. The fragmentation profile of the ions formed is the fingerprint of each methyl derivative, giving a precise analysis of the linkages, proportion of branching points, and non-reducing end units.

4.4. FT-IR spectroscopy

A very common technique in the carbohydrate analysis is the Fourier-transform infrared spectroscopy (FT-IR), which gives absorption spectra that provide information on numerous compounds, including quantitative, qualitative, physical, and chemical information related to individual components. The spectra indicate the presence of proteins, fatty acids, carbohydrates, nucleic acids, and lipopolysaccharides by the detection of NH, CO, CC, CH, CH_2, OH, and other chemical groups (Černá et al., 2003; Synytsya & Novak, 2014). Therefore, the wavenumber positions of absorbance bands are specific to the functional groups in a sample, and each

sample has a unique "fingerprint" absorbance spectrum. The intensity of absorption is directly proportional to the concentration of the absorbing compound. By these data is possible to determine which anomeric configuration and glycosidic linkage type is present, although it is not possible to define which monosaccharide is present. As well as NMR analysis, the FT-IR spectroscopy do not distinguish the presence of one or more polysaccharides in the sample, therefore, other techniques should be used to determine the homogeneity of the sample (Černá et al., 2003; Synytsya & Novak, 2014).

4.5. NMR spectroscopy

Considering that the methylation analysis shows the linkage types, but does not give information on the anomeric configuration and ring conformation, the NMR is a must use tool on the carbohydrate analysis. By the NMR experiments, it is possible to confirm the methylation data, and attribute the position of branches, as well as their proportions. The α and β configurations are easily determined by their coupling constants (Perlin & Casu, 1969; Sassaki, Gorin, de Souza, Czelusniak, & Iacomini, 2005). NMR data, when well interpreted, are very precisely because the spectra obtained are a fingerprint of the analyzed molecule. The weak points of this technique are the necessity of high amounts of sample (around 20 to 100 mg), it requires deuterium solvents, which are expensive, and it needs a highly soluble sample on the chosen solvent. In the case of D-glucans, sometimes it is difficult to dissolve them in water, because they can form gel solutions, then the dimethyl sulfoxide is the most indicated solvent. Besides, the presence of more than one polysaccharide in the sample can give erroneous assignments, considering that the NMR will not show information on the purity of the polysaccharide, therefore it is very important to use other technique to confirm the homogeneity of the sample.

Another point that should be considered is the chemical shift reference standards. Although NMR data referenced to tetramethylsilane (TMS) have been reported for solutions of carbohydrates the most common internal references used for the NMR analysis of D-glucans are: acetone, dimethyl sulfoxide-d_6, and 4,4-dimethyl-4-silapentane-1-sulphonate (DSS). Literature values of ^{13}C for acetone vary from δ 30.2 (Santos-Neves et al., 2008a; Ruthes et al., 2013b; Smiderle et al., 2013), δ 31.05 (Mandal et al., 2010; Nandi et al., 2014), δ 31.08 (Li, Dobruchowsk, Gerwig, Dijkhuizen, & Kamerling, 2013), to δ 32.9 (Bubb, 2003). The dimethyl sulfoxide-d_6 is set at δ 39.5 (Liu, Du, Wang, Zha, & Zhang, 2014b) or at δ 39.7 (Amaral et al., 2008; Carbonero et al., 2012), while DSS is 0.0 ppm (Klaus et al., 2011). The variations of the internal standards and their values can cause some confusion for comparative studies and this issue should be taken into consideration to improve the carbohydrate analysis.

4.6. Periodate oxidation and controlled Smith degradation

The determination of the main chain of polysaccharides usually requires chemical approaches to remove the branches or break the polymers in oligosaccharides. The most common mushroom D-glucan characterized present a main chain composed of β-D-Glc*p* units (1 → 3)-linked, some of them being substituted at O-6 by β-D-Glc*p* single units or oligosaccharides (Zhang et al., 2011; Zhang, Konga, Fang, Nishinari, & Phillips, 2013). The anomeric configuration can be determined by NMR techniques, although the main chain structure is more precisely defined after removing the branches. In the case of the common β-D-glucan (1 → 3)-linked branched at O-6 position, the main chain is resistant to periodate oxidation, while the (1 → 6)-linked β-D-Glc*p*, and non-reducing end units are oxidized and easily removed after mild hydrolysis (Abdel-Akher, Hamilton, Montgomery, & Smith, 1952; Hay, Lewis, & Smith, 1965; Goldstein, Hay, Lewis, & Smith, 2005). The periodate oxidation reaction is very important to define if the resistant (1 → 3)-linkages are part of the backbone structure or if they are present also in the branches, or as an alternate main chain, containing (1 → 6) and (1 → 3)-linkages.

The Controlled Smith degradation is a simple method that provides a lot of information about the chemical structure of the polysaccharide. The literature have shown many studies using this approach to determine the structure of D-glucans (Smiderle et al., 2006; Santos-Neves et al., 2008a; Liu et al., 2012; Bhanja et al., 2014), although the results obtained after this reaction and after mild hydrolysis should be carefully interpreted to avoid ambiguous chemical structures.

5. Structural features

Linear D-glucans were observed for some species of Basidiomycetes (Table 1) such as α-D-glucan (1 → 3)-linked (Nie, Zhang, Li, & Xie, 2013; Bhanja et al., 2014), α-D-glucan (1 → 4)-linked (Palacios et al., 2012), β-D-glucan (1→3)-linked (Alquini, Carbonero, Rosado, Cosentino, & Iacomini, 2004; Chakraborty, Mondal, Rout, & Islam, 2006), and β-D-glucan (1 → 6)-linked (Li et al., 2013; Smiderle et al., 2013). These linear structures were obtained from edible mushrooms as *Agaricus brasiliensis*, *Agaricus biporus*, *Coprinus comatus*, *Laetiporus sulphureus*, *Pleurotus ostreatus*, *Ramaria botrytis*, and *Termitomyces eurhizus*, mainly by hot water extraction, although they were purified using different purification procedures (Table 1).

The most common D-glucan isolated from Basidiomycetes described in the literature is a β-D-glucan with a (1 → 3)-linked main chain substituted at O-6 by single units of β-D-glucopyranose (Carbonero et al., 2006; Smiderle et al., 2006; Santos-Neves et al., 2008b; Smiderle et al., 2008a; Zhang, 2009; Zhang et al., 2011; Carbonero et al., 2012; Palacios et al., 2012; Lam & Cheung, 2013; Ruthes et al., 2013b; Zhang et al., 2013; Bhanja et al., 2014; Giavasis, 2014; Liu et al., 2014a). The previous interest on these β-D-glucans was based on a marked therapeutic property, such as the antitumoral effect initially observed for lentinan, schizophyllan, and grifolan (Zhang, Cui, Cheung, & Wang, 2007; Zhang et al., 2011, 2013). The difference observed among these β-D-glucans is the degree of branching that usually depends on the source from where the D-glucan was isolated. This can be observed on Table 2. The main problem observed in the literature about D-glucan nomenclature is the poor description of the branches. For example, the branched (1 → 3), (1 → 6)-β-D-glucan is frequently nominated as (1 → 3) β-D-glucan, which may confuse the readers and give mistaken information of a linear β-D-glucan. The polysaccharides should be described in details and as much as information about its structure should be provided to avoid further misinterpretation of the data.

Another similar β-D-glucan was observed for *Amanita muscaria* (Ruthes et al., 2013a), *Lentinus edodes* (Liu et al., 2014b), *Schizophyllum commune* (Giavasis, 2014), and *Lactarius rufus* (Ruthes et al., 2013b). The main chain is composed by β-D-Glc*p*-(1 → 3)-linked, some of them substituted at O-6 by side branches formed by β-D-Glc*p* (1 → 3)- or (1 → 6)-linked (Table 3).

Glycogen-like structures were also reported for *C. comatus* (Li et al., 2013) and *Agaricus bisporus* (Smiderle et al., 2010). The main chain was composed by α-D-glucopyranose (1 → 4)-linked units poorly substituted at O-6 by α-D-Glc*p* units or α-D-Glc*p* (1 → 6)-linked groups. Mandal et al. (2010) characterized other variations: one D-glucan containing a backbone of (1 → 6) β-D-Glc*p* substituted at O-4 by α-D-Glc*p* units, in the proportion of 2:1; and a second D-glucan with a backbone of (1 → 3) β-D-Glc*p* substituted at O-4 by β-D-Glc*p* units (Mandal et al., 2010).

Table 1
Isolation, structure and molecular weight of some linear D-glucans.

Linear Glucans				
Source	Fraction/extraction	Structure residue	M_w	Reference
Agaricus bisporus	Hot water extract[1]	(1 → 6) β-D-glucan	2.9×10^4 g mol^{-1}	Smiderle et al. (2013)
Agaricus brasiliensis (=blazei)	Hot water extract[1]	(1 → 6) β-D-glucan	4.5×10^4 g mol^{-1}	Smiderle et al. (2013)
Coprinus comatus	Hot water extract[2]	(1 → 6) β-D-glucan		Li et al. (2013)
Ganoderma lucidum	Alkali extract[3]	(1 → 3) α-D-glucan		Nie et al. (2013)
Laetiporus sulphureus	Hot water extract[4]	(1 → 3) β-D-glucan		Alquini et al. (2004)
Pleurotus ostreatus	Hot water extract[5]	(1 → 4) α-D-glucan	140,000 Da	Palacios et al. (2012)
Ramaria botrytis	Hot water extract[6]	(1 → 3) α-D-glucan		Bhanja et al. (2014)
Termithomyces eurhizus	Alkali extract[7]	(1 → 3) β-D-glucan		Chakraborty et al. (2006)

[1] After purification steps, as ethanol precipitation, freeze–thawing, dimethylsulfoxide treatment, and dialysis.
[2] After purification steps, as ethanol precipitation, DEAE-Sepharose CL-6B weak anion-exchange chromatography, and Sepharose CL-4B gel-permeation chromatography.
[3] Extraction at 25 °C and 65 °C.
[4] After purification steps, as ethanol precipitation and freeze–thawing.
[5] After purification steps, as precipitation with 20% TCA, treatment with NaCl 1%, and ethanol precipitation.
[6] After purification steps, as 5% NaOH treatment, followed by neutralization with AcOH (pH 7.0), and centrifugation.
[7] After purification steps, as ethanol precipitation, dialysis, and re-precipitation with EtOH.

Other D-glucan structures proposed in the literature are described in Table 3. Although the most original D-glucan structures, containing various combinations of configuration and linkages in the same structure should be carefully revised about the possibility of a contamination with other D-glucans in its content. The great importance of the purification methods and verification of homogeneity of the samples are closely related to a reliable result and structure description. Some articles have provided full polysaccharide NMR details, although they failed to present purification data and homogeneity analysis of the samples, which may invalidate their results. The polysaccharide analysis, as any other research field, requires further commitment of the scientists, with the aim of improving the advances in medicine and industry.

6. Biological activities

Mushroom polysaccharide extracts have been extensively studied as therapeutics for the treatment of many diseases. These fungi have been increasingly consumed as dietary supplements by cancer patients, during their treatments (Moradali et al., 2007; Hardy, 2008). The D-glucans are the main components of these extracts and, probably are mainly responsible for the medicinal effects observed. Among the mushroom extracts, the most used are derived from *S. commune*, *Ganoderma lucidum*, *A. brasiliensis*, *L. edodes*, *Trametes versicolor*, and *Grifola frondosa* (Giavasis, 2014). All the mentioned mushrooms contain D-glucans, especially the β-D-glucans, because these polymers are the basis of fungal cell wall structure (Zhang et al., 2011; Nie et al., 2013; Zhang et al., 2013; Giavasis, 2014).

There are a plenty of studies from *in vitro* and *in vivo* experiments that showed some biological properties of mushroom β-D-glucans. Some examples are: the antitumor (Zhang et al., 2011; Ren, Perera, & Hemar, 2012), anti-oxidative (Liu et al., 2014b; Nandi et al., 2014), anti-inflammatory (Dore et al., 2007; Smiderle et al., 2008a; Ruthes et al., 2013a,b), antinociceptive (Smiderle et al., 2008a; Ruthes et al., 2013b) and immunomodulatory (Lull, Wichers, & Savelkoul, 2005; Roy et al., 2009; Smiderle et al., 2013) activities.

Differences have been reported for immunostimulating and antitumoral activities of (1 → 3) β-D-glucans when compared to (1 → 4) and (1 → 6) β-D-glucans. This may be explained by

Table 2
Isolation, structure and molecular weight of some branched β-D-glucans substituted by single glucose units.

Source	Fraction/Extraction	Main Chain Residue	Branch Residue	M_w	Reference
Flammulina velutipes	Alkali extract[1]	→3)-β-D-Glc*p*-(1→[2]	β-D-Glc*p*		Smiderle et al. (2006)
Ganoderma lucidum	Hot water extract[3]	→3)-β-D-Glc*p*-(1→[4]	β-D-Glc*p*	3.75×10^6 Da	Liu et al. (2014a,b)
Grifola frondosa		→3)-β-D-Glc*p*-(1→[4]	β-D-Glc*p*		Giavasis (2014); Lam and Cheung (2013)
Lactarius rufus	Hot water extract[5]	→3)-β-D-Glc*p*-(1→[2]	β-D-Glc*p*		Ruthes et al. (2013a)
Lentinus edodes	Hot water extract and Alkali extract	→3)-β-D-Glc*p*-(1→[6]	β-D-Glc*p*	$3.0–18 \times 10^5$	Giavasis (2014), Zhang et al. (2011)
Pleurotus eryngii	Hot water extract[5]	→3)-β-D-Glc*p*-(1→[4]	β-D-Glc*p*		Carbonero et al. (2006)
Pleurotus florida	Alkali extract[1]	→3)-β-D-Glc*p*-(1→[2]	β-D-Glc*p*	1.2×10^6 g mol^{-1}	Santos-Neves et al. (2008b)
Pleurotus geestanus	Hot water extract[7]	→3)-β-D-Glc*p*-(1→[4]	β-D-Glc*p*	40.0×10^4	Zhang (2009)
Pleurotus ostreatoroseus	Hot water extract[5]	→3)-β-D-Glc*p*-(1→[4]	β-D-Glc*p*		Carbonero et al. (2006)
Pleurotus ostreatus	Alkali extract[8]	→3)-β-D-Glc*p*-(1→[2]	β-D-Glc*p*	30,000 Da	Lam and Cheung (2013), Giavasis (2014)
		→3)-β-D-Glc*p*-(1→[9]	β-D-Glc*p*		Palacios et al. (2012)
Pleurotus pulmonarius	Hot water extract[10]	→3)-β -D-Glc*p*-(1→[2]	β-D-Glc*p*		Smiderle et al. (2008b)
Ramaria botrytis	Alkali extract[11]	→3)-β -D-Glc*p*-(1→[2]	β-D-Glc*p*		Bhanja et al. (2014)
Schizophyllum commune		→3)-β -D-Glc*p*-(1→[2]	β-D-Glc*p*		Lam and Cheung (2013), Zhang et al. (2013)

[1] After purification steps, as neutralization with AcOH, dialysis, and freeze–thawing.
[2] Substituted at O-6, each fourth residue of the main chain.
[3] After purification steps, as ethanol precipitation.
[4] Substituted at O-6, each third residue of the main chain.
[5] After purification steps, as ethanol precipitation, freeze–thawing, and centrifugation.
[6] Two substitutions at O-6, every five residues of the main chain.
[7] After purification steps, as ethanol precipitation, followed by 15-20% ammonium sulfate precipitation.
[8] After purification steps, as precipitation with 20% TCA, treatment with NaCl 1%, and ethanol precipitation.
[9] Substituted at O-6, each 15th residue of the main chain.
[10] After purification steps, as ethanol precipitation, freeze–thawing, and hot 2% aq. NaOH treatment.
[11] After purification steps, as 5% NaOH treatment, followed by neutralization with AcOH (pH 7.0), centrifugation, and dialysis.

Table 3
Isolation, structure and molecular weight of some branched D-glucans substituted by different side chains.

Source	Fraction/Extraction	Main Chain Residue	Branch Residue	M_w	Reference
Agaricus bisporus	Hot water extract[1]	→4)-α-D-Glc*p*-(1→[2]	α-D-Glc*p*		Smiderle et al. (2010)
Amanita muscaria	Alkali extract[3]	→3)-β-D-Glc*p*-(1→[4]	β-D-Glc*p* or →3)-β-D-Glc*p*-(1→	16.2×10^3 g mol^{-1}	Ruthes et al. (2013b)
Calocybe indica	Alkali extract[5]	→6)-β-D-Glc*p*-(1→[6]	α-D-Glc*p*	~1.87×10^5 Da	Mandal et al.
	Alkali extract[7]	→3)-β-D-Glc*p*-(1→[8]	β-D-Glc*p*	~2.0×10^5 Da	(2010)
Coprinus comatus	Hot water extract[9]	→4)-α-D-Glc*p*-(1→[10]	α-D-Glc*p* or →6)-α-D-Glc*p*-(1→		Li et al. (2013)
Entoloma lividoalbum	Alkali extract[5]	→6)-β-D-Glc*p*-(1→[11]	→3)-β-D-Glc*p*-(1→	~2.1×10^5 Da	Maity et al. (2014)
Ganoderma resinaceum	Alkali extract[12]	→3)-β-D-Glc*p*-(1→[13]	→4)-β-D-Glc*p*-(1→	2.6×10^4 g mol^{-1}	Amaral et al. (2008)
Lactarius rufus	Hot water extract[3]	→3)-β-D-Glc*p*-(1→[14]	β-D-Glc*p* or →3)-β-D-Glc*p*-(1→	11.3×10^4 g mol^{-1}	Ruthes et al. (2013a)
Lentinus edodes	Alkali extract[15]	→3)-β-D-Glc*p*-(1→[16]	→6)-β-D-Glc*p*-(1→	~2.02×10^5 Da	Liu et al. (2014a,b)
Macrolepiota dolichaula	Hot water extract[5]	→6)-β-D-Glc*p*-(1→[11]	β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→		Samanta et al. (2013)
Pleurotus citrinopileatus	Hot water extract[17]	→6)-β-D-Glc*p*-(1→[18]	β-D-Glc*p* or →3)-β-D-Glc*p*-(1→	45,000 Da	Liu et al. (2012)
Pleurotus florida	Cold and Hot water extract[3]	→3)-α-D-Glc*p*-(1→[4]	β-D-Glc*p* or →3)-β-D-Glc*p*-(1→	1.1×10^6 g mol^{-1}	Santos-Neves et al. (2008a)
Russula albonigra	Alkali extract[5]	→6)-β-D-Glc*p*-(1→[11]	→3)-β-D-Glc*p*-(1→	~1.95×10^5 Da	Nandi et al. (2014)

[1] After purification steps, as ethanol precipitation, and DEAE cellulose chromatography.
[2] Substituted at O-6, each ninth residue of the main chain.
[3] After purification steps, as freeze–thawing, treatment with Fehling solution, and centrifugation.
[4] Mostly substituted at O-6 by single units, and partially by side chains.
[5] After purification steps, as ethanol precipitation, dialysis, and fractionation through Sepharose 6B column.
[6] Substituted at O-4, each third residue of the main chain.
[7] After purification steps, as ethanol precipitation, dialysis, and centrifugation.
[8] Substituted at O-4, each fourth residue of the main chain.
[9] After purification steps, as ethanol precipitation, treatment with 0.2 M PBS, and fractionation through DEAE-Sepharose CL-6B column.
[10] Approximately 10% of branching at O-6.
[11] Substituted at O-3, each third residue of the main chain.
[12] After purification steps, as freeze–thawing, centrifugation, dialysis, and ultrafiltration through 10 kDa *cut-off* membrane.
[13] Substituted at O-6, on the average of one to every two residues of the backbone.
[14] Substituted at O-6, in the proportion of 1:1.2.
[15] After purification steps, as neutralization, centrifugation, and deproteinization by Sevag method.
[16] Substituted at O-6.
[17] After purification steps, as DEAE-Sepharose Fast Flow, and Sephadex G-200 gel-permeation chromatography.
[18] Substituted at O-3, each fourth residue of the main chain.

physical-chemical differences, although the relationship between structure and medicinal property requires more investigation (Thompson, Oyston, & Williamson, 2010; Lehtovaara & Gu, 2011; Ren et al., 2012). Some authors have showed that the D-glucans primary structure, molecular weight, branching degree, and quaternary structure are important for the bioactivity of these compounds (Goodridge et al., 2011; Lehtovaara & Gu, 2011). Studies have shown that soluble and insoluble β-D-glucans are able to bind to dectin-1 receptor, although only the insoluble (particulate) ones were able to induce phagocytosis activity (Goodridge et al., 2011). Lentinan, the (1 → 3),(1 → 6) β-D-glucan isolated from Shiitake (*L. edodes*), in its triple-helical conformation inhibited the growth of solid tumors (sarcoma-180). This inhibitory effect was not observed when the tumors were treated by the single-helical polysaccharide (Zhang, Li, Xua, & Zeng, 2005). The presence of (1 → 3)-linkages in the main chain, and of (1 → 6)-linkages on the branches seems to be required for the antitumoral activity. The degree of these branches was also reported to show different effects, as poorly branched D-glucans, presenting a degree of branching (DB) between 0.20 and 0.33, were the most potent antitumor homopolysaccharides, while the D-glucans with higher DB (0.67 and 0.75) were not effective (Lehtovaara & Gu, 2011; Zhang et al., 2013).

There is a lot of information on the biological effects exhibited by mushroom D-glucans, although the majority of the studies are still performed using the mushroom extracts, which contain D-glucans plus other compounds. Therefore it is required more details on complete purification and chemical characterization of these D-glucans, with the aim of determining the correlation between their chemical structures and therapeutic effects.

7. Potential applications and future perspectives

The growing interest in renewable resources as well as to the wide applications of polysaccharides in medicine and food fields, such as the immunostimulatory and anti-tumor effects, and no toxicity has been prompted researchers out to investigate these polymers structure trying to establish a structure-activity relationship. However, despite the increase in research in this direction, little is known about the structural diversity of polysaccharides and how it affects their possible applications.

Besides being a renewable natural source of non-animal origin, another advantage presented by mushrooms is due to the fact that from the discovery of a polysaccharide with industrial or medical interest, it can be produced in large scale, or having its production optimized by submerged cultivation.

Most studies involving mushroom polysaccharides, evaluate only crude extracts or a mixture of polysaccharides, in which the whole process of fractionation and purification is left. Due to its predominance researchers often mistakenly assume that these extracts are composed solely of D-glucans.

D-Glucans have great potential in a wide variety of fields due to their conformation arrangement, gel-forming capacity and potent pharmacological properties. These molecules present potential use in immunotherapeutic intervention, in the food industry, and recently, it has increased their use for the development of nanostructures and delivery of active therapy. Based on this information, two strands of research can be clearly identified: one involving the pharmacological properties and other related to industrial application. Both lines of research require a higher number of scientists devoted to study the potential applications of D-glucans.

8. Conclusions

Mushrooms are viewed as an important source of bioactive polysaccharides. The most common polysaccharide found in edible mushrooms consists of D-glucans; nevertheless, other polysaccharides, such as heterogalactans or heteromannans, or even polysaccharide-protein complexes can also be found.

D-Glucans have been considered as biological response modifiers due to their ability to enhance the immune system and, therefore, prevent and treat several common diseases and promote health.

In the last few decades, the novel potential applications attributed to polysaccharides have provided a major impetus that increased scientific attention. Among the most promising aspects are their immunomodulatory and antitumor effects, thickening characteristic and stabilizer effects. It is well known that the molecular weight, conformation, chemical modification and solubility of the polysaccharides significantly affect their activities. Thus, the purification and determination of the molecular weight of polymers becomes very important for a possible pharmacological or industrial application.

Acknowledgments

The authors would like to thank the Brazilian funding agencies: CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and Fundação Araucária for financial support. A.C. Ruthes is beneficiary of post-doctoral scholarship from CAPES-PNPD (02675/09-9); F.R. Smiderle is beneficiary of post-doctoral scholarship from CNPq (150340/2014-1).

References

Abdel-Akher, M., Hamilton, J. K., Montgomery, R., & Smith, F. (1952). A new procedure for the determination of the fine structure of polysaccharides. *Journal of the American Chemical Society*, *74*, 4970–4971.

Alquini, G., Carbonero, E. R., Rosado, F. R., Cosentino, C., & Iacomini, M. (2004). Polysaccharides from the fruit bodies of the basidiomycete *Laetiporus sulphureus* (Bull.: Fr.) Murr. *FEMS Microbiology Letters*, *230*, 47–52.

Amaral, A. E., Carbonero, E. R., Simão, R. C. G., Kadowaki, M. K., Sassaki, G. L., Osaku, C. A., et al. (2008). An unusual water-soluble β-glucan from the basidiocarp of the fungus *Ganoderma resinaceum*. *Carbohydrate Polymers*, *72*, 473–478.

Bae, I. Y., Kim, H. W., Yoo, H. J., Kim, E. S., Lee, S., Park, D. Y., et al. (2013). Correlation of branching structure of mushroom β-glucan with its physiological activities. *Food Research International*, *51*, 195–200.

Bhanja, S. K., Rout, D., Patra, P., Sena, I. K., Nandan, C. I. K., & Islam, S. S. (2014). Water-insoluble glucans from the edible fungus *Ramaria botrytis*. *Bioactive Carbohydrate Dietary Fiber*, *3*, 52–58.

Bi, H., Ni, X., Liu, X., Iteku, J., Tai, G., Zhou, Y., et al. (2009). A novel water-soluble β-(1,6)-D-glucan isolated from the fruit bodies of *Bulgaria inquinans* (Fries). *Carbohydrate Research*, *344*, 1254–1258.

Bubb, W. A. (2003). NMR spectroscopy in the study of carbohydrates: Characterizing the structural complexity. *Concepts in Magnetic Resonance, A*, *19A*, 1–19.

Carbonero, E. R., Gracher, A. H. P., Smiderle, F. R., Rosado, F. R., Sassaki, G. L., Gorin, P. A. J., et al. (2006). A β-glucan from the fruit bodies of edible mushrooms *Pleurotus eryngii* and *Pleurotus ostreatoroseus*. *Carbohydrate Polymers*, *66*, 252–257.

Carbonero, E. R., Gracher, A. H. P., Komura, D. L., Marcon, R., Freitas, C. S., Baggio, C. H., et al. (2008). *Lentinus edodes* heterogalactan: Antinociceptive and anti-inflammatory effects. *Food Chemistry*, *111*, 531–537.

Carbonero, E. R., Ruthes, A. C., Freitas, C. S., Utrilla, P., Gálvez, J., da Silva, E. V., et al. (2012). Chemical and biological properties of a highly branched β-glucan from edible mushroom *Pleurotus sajor-caju*. *Carbohydrate Polymers*, *90*, 814–819.

Černá, M., Barros, A. S., Nunes, A., Rocha, S. M., Delgadillo, I., ČopííKová, J., et al. (2003). Use of FT-IR spectroscopy as a tool for the analysis of polysaccharide food additives. *Carbohydrate Polymers*, *51*(4), 383–389.

Chakraborty, I., Mondal, S., Rout, D., & Islam, S. S. (2006). A water-insoluble (1,3)-β-D-glucan from the alkaline extract of an edible mushroom *Termitomyces eurhizus*. *Carbohydrate Research*, *341*, 2990–2993.

Chen, X., Zhang, L., & Cheung, P. C. K. (2010). Immunopotentiation and anti-tumor activity of carboxymethylated-sulfated β-(1→3)-D-glucan from Poria cocos. *International Immunopharmacology*, *10*, 398–405.

Chen, W., Wang, W.-P., Zhang, H.-S., & Huang, Q. (2012). Optimization of ultrasonic-assisted extraction of water-soluble polysaccharides from *Boletus edulis* mycelia using response surface methodology. *Carbohydrate Polymers*, *87*(1), 614–619.

Cheung, P. C. K. (2013). Mini-review on edible mushrooms as source of dietary fiber: Preparation and health benefits. *Food Science and Human Wellness*, *2*, 162–166.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, *131*, 209–217.

Dore, C. M. P. G., Azevedo, T. C. G., de Souza, M. C. R., Rego, L. A., de Dantas, J. C. M., Silva, R. F., et al. (2007). Antiinflammatory, antioxidant and cytotoxic actions of β-glucan-rich extract from Geastrum saccatum mushroom. *International Immunopharmacology*, *7*, 1160–1169.

Dong, Q., Wang, Y., Shi, L., Yao, J., Li, J., Ma, F., et al. (2012). A novel water-soluble β-D-glucan isolated from the spores of *Ganoderma lucidum*. *Carbohydrate Research*, *353*, 100–105.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Giavasis, I. (2014). Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. *Current Opinion in Biotechnology*, *26*, 162–173.

Goldstein, I. J., Hay, G. W., Lewis, B. A., & Smith, F. (2005). Controlled degradation of polysaccharides by periodate oxidation, reduction, and hydrolysis. *Methods in Carbohydrate Chemistry*, *5*, 361–369.

Goodridge, H. S., Reyes, C. N., Becker, C. A., Katsumoto, T. R., Ma, J., Wolf, A. J., et al. (2011). Activation of the innate immune receptor Dectin-1 upon formation of a 'phagocytic synapse'. *Nature*, *472*, 471–475.

Gorin, P. A. J., & Iacomini, M. (1984). Polysaccharides of the lichens *Cetraria islandica* and Ramalina usnea*. *Carbohydrate Research*, *128*, 119–132.

Hardy, M. L. (2008). Dietary supplement use in cancer care: Help or harm. *Hematology/Oncology Clinics of North America*, *22*, 581–617.

Haworth, W. N. (1915). A new method of preparing alkylated sugars. *Journal of the Chemical Society, Transactions*, *107*, 8–16.

Hay, G. W., Lewis, B. A., & Smith, F. (1965). Periodate oxidation of polysaccharides: General procedures. *Methods in Carbohydrate Chemistry*, *5*, 357–360.

Jones, J. K. N., & Stoodley, R. J. (1965). Fractionation using copper complexes. *Methods in Carbohydrate Chemistry*, *5*, 36–38.

Kalac, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, *113*, 9–16.

Klaus, A., Kozarski, M., Niksic, M., Jakovljevic, D., Todorovic, N., & Van Griensven, L. J. L. D. (2011). Antioxidative activities and chemical characterization of polysaccharides extracted from the basidiomycete *Schizophyllum commune*. *LWT Food Science and Technology*, *44*, 2005–2011.

Komura, D. L., Carbonero, E. R., Gracher, A. H. P., Baggio, C. H., Freitas, C. S., Marcon, R., et al. (2010). Structure of Agaricus spp. fucogalactans and their anti-inflammatory and antinociceptive properties. *Bioresource Technology*, *101*, 6192–6199.

Lam, K. L., & Cheung, P. C. K. (2013). Non-digestible long chain beta-glucans as novel prebiotics. *Bioactive Carbohydrate and Dietary Fiber*, *2*, 45–64.

Lehtovaara, B. C., & Gu, F. X. (2011). Pharmacological, structural, and drug delivery properties and applications of 1,3-β-glucans. *Journal of Agricultural and Food Chemistry*, *59*, 6813–6828.

Li, B., Dobruchowsk, J. M., Gerwig, G. J., Dijkhuizen, L., & Kamerling, J. P. (2013). Structural investigation of water-soluble polysaccharides extracted from the fruit bodies of *Coprinus comatus*. *Carbohydrate Polymers*, *91*, 314–321.

Liu, J., Sun, Y., Yu, H., Zhang, C., Yue, L., Yang, X., et al. (2012). Purification and identification of one glucan from golden oyster mushroom (*Pleurotus citrinopileatus* (Fr.) Singer). *Carbohydrate Polymers*, *87*, 348–352.

Liu, Y., Zhang, J., Tang, Q., Yang, Y., Guo, Q., Wang, Q., et al. (2014). Physicochemical characterization of a high molecular weight bioactive β-D-glucan from the fruiting bodies of *Ganoderma lucidum*. *Carbohydrate Polymers*, *101*, 968–974.

Liu, Y., Du, Y. Q., Wang, J. H., Zha, X. Q., & Zhang, J. B. (2014). Structural analysis and antioxidant activities of polysaccharide isolated from Jinqian mushroom. *International Journal of Biological Macromolecules*, *64*, 63–68.

Lull, C., Wichers, J., & Savelkoul, H. F. J. (2005). Antiinflammatory and immunomodulating properties of fungal metabolites. *Mediators of Inflammation*, *2*, 63–80.

Maity, P., Samanta, S., Nandi, A. K., Sen, I. K., Paloi, S., Acharya, K., et al. (2014). Structure elucidation and antioxidant properties of a soluble β-D-glucan from mushroom *Entoloma lividoalbum*. *International Journal of Biological Macromolecules*, *63*, 140–149.

Mandal, S., Maity, K. K., Bhunia, S. K., Dey, B., Patra, S., Sikdar, S. R., et al. (2010). Chemical analysis of new water-soluble (1,6)-, (1,4)-α,β-glucan and water-insoluble (1,3)-, (1,4)-β-glucan (Calocyban) from alkaline extract of an edible mushroom, *Calocybe indica* (Dudh Chattu). *Carbohydrate Research*, *345*, 2657–2663.

Moradali, M. F., Mostafavi, H., Ghods, S., & Hedjaroude, G. A. (2007). Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). *International Immunopharmacology*, *7*, 701–724.

Nandi, A. K., Samanta, S., Maity, S., Sen, I. K., Khatua, S., Devi, K. S. P., et al. (2014). Antioxidant and immunostimulant β-glucan from edible mushroom *Russula albonigra* (Krombh.) Fr. *Carbohydrate Polymers*, *99*, 774–782.

Nie, S., Zhang, H., Li, W., & Xie, M. (2013). Current development of polysaccharides from Ganoderma: Isolation, structure and bioactivities. *Bioactive Carbohydrate and Dietary Fiber*, *1*, 10–20.

Palacios, I., Garcia-Lafuente, A., Guillamón, E., & Villares, A. (2012). Novel isolation of water-soluble polysaccharides from the fruiting bodies of *Pleurotus ostreatus* mushrooms. *Carbohydrate Research*, *358*, 72–77.

Palanisamy, M., Aldars-García, L., Gil-Ramírez, A., Ruiz-Rodríguez, A., Marín, F. R., Reglero, G., et al. (2014). Pressurized water extraction of β-glucan enriched fractions with bile acids-binding capacities obtained from edible mushrooms. *Biotechnology Progress*, *30*(2), 391–400.

Perlin, A. S., & Casu, B. (1969). Carbon-13 and proton magnetic resonance spectra of D-glucose-^{13}C. *Tetrahedron Letters*, *34*, 2919–2924.
Ren, L., Perera, C., & Hemar, Y. (2012). Antitumor activity of mushroom polysaccharides: A review. *Food & Function*, *3*, 1118–1130.
Roy, S. K., Das, D., Mondal, S., Maiti, D., Bhunia, B., Maiti, T. K., et al. (2009). Structural studies of an immunoenhancing water-soluble glucan isolated from hot water extract of an edible mushroom, Pleurotus florida, cultivar Assam Florida. *Carbohydrate Research*, *344*, 2596–2601.
Ruthes, A. C., Komura, D. L., Carbonero, E. R., Sassaki, G. L., Gorin, P. A. J., & Iacomini, M. (2010). Structural characterization of the uncommon polysaccharides obtained from *Peltigera canina* photobiont *Nostoc muscorum*. *Carbohydrate Polymers*, *81*, 29–34.
Ruthes, A. C., Rattmann, Y. D., Carbonero, E. R., Gorin, P. A. J., & Iacomini, M. (2012). Structural characterization and protective effect against murine sepsis of fucogalactans from *Agaricus bisporus* and *Lactarius rufus*. *Carbohydrate Polymers*, *87*, 1620–1627.
Ruthes, A. C., Carbonero, E. R., Córdova, M. M., Baggio, C. H., Sassaki, G. L., Gorin, P. A. J., et al. (2013). Fucomannogalactan and glucan from mushroom *Amanita muscaria*: Structure and inflammatory pain inhibition. *Carbohydrate Polymers*, *98*, 761–769.
Ruthes, A. C., Carbonero, E. R., Córdova, M. M., Baggio, C. H., Santos, A. R. S., Sassaki, G. L., et al. (2013). *Lactarius rufus* (1→3),(1→6)-β-D-glucans: Structure, antinociceptive and anti-inflammatory effects. *Carbohydrate Polymers*, *94*, 129–136.
Ruthes, A. C., Rattmann, Y. D., Malquevicz-Paiva, S. M., Carbonero, E. R., Córdova, M. M., Baggio, C. H., et al. (2013). *Agaricus bisporus* fucogalactan: Structural characterization and pharmacological approaches. *Carbohydrate Polymers*, *92*, 184–191.
Samanta, S., Nandi, A. K., Sen, I. K., Maji, P. K., Devi, K. S. P., Maiti, T. K., et al. (2013). Structural characterization of an immunoenhancing glucan isolated from a mushroom *Macrolepiota dolichaula*. *International Journal of Biological Macromolecules*, *61*, 89–96.
Santos-Neves, J. C., Pereira, M. I., Carbonero, E. R., Gracher, A. H. P., Alquini, G., Gorin, P. A. J., et al. (2008). A novel branched αβ-glucan isolated from the basidiocarps of the edible mushroom Pleurotus florida. *Carbohydrate Polymers*, *73*, 309–314.
Santos-Neves, J. C., Pereira, M. I., Carbonero, E. R., Gracher, A. H. P., Gorin, P. A. J., Sassaki, G. L., et al. (2008). A gel-forming β-glucan isolated from the fruit bodies of the edible mushroom Pleurotus florida. *Carbohydrate Research*, *343*, 1456–1462.
Sassaki, G. L., Gorin, P. A. J., de Souza, L. M., Czelusniak, P. A., & Iacomini, M. (2005). Rapid synthesis of partially *O*-methylated alditol acetate standards for GC–MS: Some relative activities of hydroxyl groups of methyl glycopyranosides on Purdie methylation. *Carbohydrate Research*, *340*, 731–739.
Sassaki, G. L., de Souza, L. M., Cipriani, T. R., & Iacomini, M. (2008). In M. Waksmundzka-Hajnos, J. Sherma, & T. Kowalska (Eds.), *TLC of carbohydrates in thin layer chromatography in phytochemistry* (pp. 255–256). CRC Press.
Sassaki, G. L., de Souza, L. M., Serrato, R. V., Cipriani, T. R., Gorin, P. A. J., & Iacomini, M. (2008). Application of acetate derivatives for gas chromatography mass spectrometry: Novel approaches on carbohydrates, lipids and amino acids analysis. *Journal of Chromatography A*, *1208*, 215–222.
Smiderle, F. R., Carbonero, E. R., Mellinger, C. G., Sassaki, G. L., Gorin, P. A. J., & Iacomini, M. (2006). Structural characterization of a polysaccharide and a β-glucan isolated from the edible mushroom *Flammulina velutipes*. *Phytochemistry*, *67*, 2189–2196.
Smiderle, F. R., Olsen, L. M., Carbonero, E. R., Baggio, C. H., Freitas, C. S., Marcon, R., et al. (2008). Anti-inflammatory and analgesic properties in a rodentmodel of a (1→3),(1→6)-linked β-glucan isolated from *Pleurotus pulmonarius*. *European Journal of Pharmacology*, *597*, 86–91.
Smiderle, F. R., Carbonero, E. R., Sassaki, G. L., Gorin, P. A. J., & Iacomini, M. (2008). Characterization of a heterogalactan: Some nutritional values of the edible mushroom Flammulina velutipes. *Food Chemistry*, *108*, 329–333.
Smiderle, F. R., Olsen, L. M., Carbonero, E. R., Marcon, R., Baggio, C. H., Freitas, C. S., et al. (2008). A 3-*O*-methylated mannogalactan from *Pleurotus pulmonarius*: Structure and antinociceptive effect. *Phytochemistry*, *69*, 2731–2736.
Smiderle, F. R., Sassaki, G. L., Van Arkel, J., Iacomini, M., Wichers, H. J., & Van Griensven, L. J. L. D. (2010). High molecular weight glucan of the culinary medicinal mushroom *Agaricus bisporus* is an α-glucan that forms complexes with low molecular weight galactan. *Molecules*, *15*, 5818–5830.
Smiderle, F. R., Alquini, G., Tadra-Sfeir, M. Z., Iacomini, M., Wichers, H. J., & Van Griensven, L. J. L. D. (2013). *Agaricus bisporus* and *Agaricus brasiliensis* (1→6)-β-D-glucans show immunostimulatory activity on human THP-1 derived macrophages. *Carbohydrate Polymers*, *94*, 91–99.
Song, G., & Du, Q. (2012). Structure characterization and antitumor activity of an α β-glucan polysaccharide from Auricularia polytricha. *Food Research International*, *45*, 381–387.
Staub, A. M. (1965). Removal of proteins: Sevag method. *Methods in Carbohydrate Chemistry*, *5*, 5–6.
Synytsya, A., Mičková, K., Synytsya, A., Jablonský, I., Specvácek, J., Erban, V., et al. (2009). Glucans from fruit bodies of cultivated mushrooms *Pleurotus ostreatus* and *Pleurotus eryngii*: Structure and potential prebiotic activity. *Carbohydrate Polymers*, *76*, 548–556.
Synytsya, A., & Novák, M. (2013). Structural diversity of fungal glucans. *Carbohydrate Polymers*, *92*, 792–809.
Synytsya, A., & Novak, M. (2014). Structural analysis of glucans. *Annals of Translational Medicine*, *2*(2), 1–14.
Thompson, I. J., Oyston, P. C. F., & Williamson, D. E. (2010). Potential of the β-glucans to enhance innate resistance to biological agents. *Expert Review of Anti-Infective Therapy*, *8*, 339–352.
Wang, Y., Yu, Y., & Mao, J. (2009). Carboxymethylated β-glucan derived from *Poria cocos* with biological activities. *Journal of Agricultural and Food Chemistry*, *57*, 10913–10915.
Wang, Q., Chen, S., Han, L., Lian, M., Wen, Z., Jiayinaguli, T., et al. (2014). Antioxidant activity of carboxymethyl (1→3)-β-D-glucan (from the sclerotium of *Poria cocos*) sulfate (in vitro). *International Journal of Biological Macromolecules*, *69*, 229–235.
Wasser, S. P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*, *60*, 258–274.
Zeng, W.-C., Zhang, Z., Gao, H., Jia, L.-R., & Chen, W.-Y. (2012). Characterization of antioxidant polysaccharides from *Auricularia auricular* using microwave-assisted extraction. *Carbohydrate Polymers*, *89*(2), 694–700.
Zhang, L., Zhou, J., Yang, G., & Chen, J. (1998). Preparative fractionation of polysaccharides by columns packed with regenerated cellulose gels. *Journal of Chromatography A*, *816*, 131–136.
Zhang, L., Li, X., Xua, X., & Zeng, F. (2005). Correlation between antitumor activity, molecular weight, and conformation of lentinan. *Carbohydrate Research*, *340*, 1515–1521.
Zhang, M. (2009). Heating-induced conformational change of a novel b-(1,3)-D-glucan from *Pleurotus geestanus*. *Biopolymers*, *93*, 121–131.
Zhang, M., Cui, S. W., Cheung, P. C. K., & Wang, Q. (2007). Antitumor polysaccharides from mushrooms: A review on their isolation process, structural characteristics and antitumor activity. *Trends in Food Science and Technology*, *18*, 4–19.
Zhang, Y., Li, S., Wang, X., Zhang, L., & Cheung, P. C. K. (2011). Advances in lentinan: Isolation, structure, chain conformation and bioactivities. *Food Hydrocolloids*, *25*, 196–206.
Zhang, Y., Konga, H., Fang, Y., Nishinari, K., & Phillips, G. O. (2013). Schizophyllan: A review on its structure, properties, bioactivities and recent developments. *Bioactive Carbohydrate and Dietary Fiber*, *1*, 53–71.