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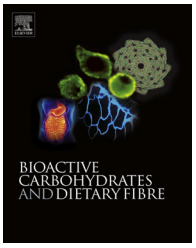

Separation and characterization of the structural features of macromolecular carbohydrates from wild edible mushrooms

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ABSTRACT

A structural characterization of two polysaccharides isolated from the fruiting bodies of the wild edible mushroom *Cantharellus cibarius* was performed after extraction and purification. Two polysaccharides were separated by successive aqueous extractions with boiling water and with NaOH aqueous solution (1 M, 100 °C), respectively. The size exclusion chromatograms from the separated fractions showed a single peak in each fraction. The polysaccharide from the boiling water fraction (PsCcib-I) was a glucan-type carbohydrate with a molecular weight of 150 kDa. The methylation analysis and NMR experiments showed that PsCcib-I was composed of a main chain consisting of α-(1→6)-Glc*p* units with β-(1→4)-linked branches every third glucose residue. Differently, the polysaccharide separated from the hot aqueous NaOH fraction (PsCcib-II) had a molecular weight of 120 kDa and appeared to be a glucan-type carbohydrate composed of β-(1→3)-linked glucose units with branches at O-6 every third sugar residue. The complexation with Congo red showed that PsCcib-II displayed a triple helical conformation.

1. Introduction

Mushrooms have been traditionally used for many years in oriental culture as health enhancers as well as in disease treatments. Among the wide range of bioactive substances occurring in mushrooms, polysaccharides comprise an important group regarding the healthy benefits of fungi. Carbohydrates from mushrooms have received considerable attention due to their demonstrated immunological, anti-inflammatory and antioxidant activities (Chen, Xie, Nie, Li, & Wang, 2008; Ruthes et al., 2013), among others, which are involved in the prevention against common human chronic diseases, such as cancer, diabetes or even cardiovascular diseases (Rop, Mlcek, & Jurikova, 2009; Stachowiak & Regula, 2012; Wang, Zhang, Yu, & Cheung, 2009).

Several polysaccharides have been previously extracted from mushrooms, being the most important the β-linked glucans, for instance, lentinan from *Lentinus edodes* (Lo, Tsao, Wang, & Chang, 2007; Zhang, Li, Wang, Zhang, & Cheung, 2011), pleuran from *Pleurotus* spp. (Karacsonyi & Kuniak, 1994), ganoderan and ganopoly from *Ganoderma lucidum* (Wang & Zhang, 2009), or schizophyllan from *Schizophillium commune* (Numata et al., 2006), among others. Furthermore, other structural features have also been described in polysaccharides from mushrooms, for instance, αβ-glucans (Song & Du, 2012), heteropolysaccharides formed by different sugars (Chen et al., 2008; Ding, Hou, & Hou, 2012; Liu et al., 2011; Smiderle, Carbonero, Sassaki, Gorin, & Iacomini, 2008), or even protein–glucan complexes, such as the protein-bound polysaccharide krestin from *Coriolus versicolor* (Kobayashi, Matsunaga, & Fujii, 1993).

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The elucidation of molecular structure of polysaccharides is a very important issue in therapeutic activity since the bioavailability may be associated to structural patterns (Bohn & BeMiller, 1995). The biological response is closely related to the molecular weight, the chemical structure, in terms of the chain composition and the occurrence and position of branches, and the three-dimensional arrangement, which influences the behavior and the physical properties like solubility, viscosity or gelation (Li, Zhang, & Xu, 2009).

New sources of bioactive polysaccharides are gaining much importance due to the possibility to obtain unique structural features which would imply biological activity. In this field, the genus *Cantharellus* has been scarcely studied and, to the best of our knowledge, the structural features of polysaccharides extracted from this mushroom have not been described yet. Despite being a wild mushroom, *Cantharellus cibarius* are widely consumed since the fruiting bodies of this edible fungus are harvested worldwide, including Europe, North America, Asia, and Africa. The aim of the present study was the isolation and characterization of polysaccharides occurring in the wild edible mushroom *C. cibarius*. This article describes, apparently for the first time, the extraction and purification processes of polysaccharides from *C. cibarius* together with the elucidation of their structural features.

2. Materials and methods

2.1. Materials and reagents

The fruiting bodies of the mushroom chanterelle (*C. cibarius*) were purchased from local supermarket. The samples were cleaned with distilled water, dried with paper and immediately freeze-dried in a lyophilizer (Telstar Cryodos, Spain). Dried mushrooms were finely milled with a M20 mill (IKA Werke, Germany) to obtain a particle size lower than 500 μm. Samples were kept at 4 °C in hermetically vacuum-sealed plastic bags up to analysis.

2.2. Extraction and purification of polysaccharides

Freeze-dried mushrooms (1 g) were firstly extracted with methanol at 65 °C for 8 h to remove soluble molecules, such as lipids, soluble sugars or phenolic compounds. First polysaccharide extraction was carried out with water at room temperature (30 mL, 24 h). The solid was separated by centrifugation at 4000 rpm (2665*g*) in a Universal 320R centrifuge (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) at 4 °C to give a clear supernatant. The residue was re-extracted with water at 100 °C (30 mL, for 24 h). The extract was centrifuged at 4000 rpm at 4 °C to give a clear supernatant, which was separated from the residue. The remainder solid was finally extracted with aqueous NaOH solutions (1 M) at 100 °C (30 mL, for 24 h) and again separated from the liquid by centrifugation. The liquids from each extraction (water at room temperature, boiling water and hot NaOH aqueous solutions, respectively) were precipitated separately by the addition of ethanol in a 2:1 ratio (*v*/*v*) and the resulting polysaccharide extracts were re-dissolved in distilled water. Further purification steps (removal of proteins and other macromolecules) were performed as previously described for polysaccharides from mushrooms with minor modifications for *C. cibarius* (Palacios, García-Lafuente, Guillamón, & Villares, 2012; Palacios, Guillamón, García-Lafuente, & Villares, 2012).

2.3. Determination of homogeneity of polysaccharides and their molar mass

The molecular weight of the polysaccharides was measured by using an Alliance® HPLC system 2695 equipped with a refractive index detector 2414 and coupled to a fraction collector III (Waters Corp., Milford, Massachusetts). Separation was achieved on a Protein-Pak 300 SW SEC column (10 μm × 30 cm; 8.0 mm i.d.; Waters Corp., Milford, Massachusetts) at 30 °C. Refractive index detector was thermostatted at 30 °C. The sample volume injection was 10 μL. The solvent was 0.1 M $NaNO_3$ aqueous solution with a flow rate of 0.2 mL/min. Dextran standards 6, 40, 70, 100, 500 and 2000 kDa (Sigma Chemical Co, St. Louis, MO, USA) were used to obtain the molecular weight standard curve. Prior to HPLC analysis, samples were dissolved in water and filtered through a 0.45 μm nylon syringe filter (VWR-International, Darmstadt, Germany).

2.4. Methylation analysis of polysaccharide fractions

Methylation of the polysaccharide was performed according to the method described by Ciucanu (2006) with minor modifications for fungal polysaccharides (Palacios et al., 2012). Briefly, 10 mg of polysaccharides was dissolved in dimethylsulfoxide overnight, and anhydrous sodium hydroxide was added. The reaction mixture was cooled and 500 μL of methyl iodide was added during stirring in an ice bath for 40 min. The reaction was stopped with water and the resulting partially methylated derivatives were extracted with chloroform and dried with anhydrous $MgSO_4$. Chloroform was evaporated under a nitrogen stream and the hydrolysis was performed with TFA in an autoclave (Trade Raypa) at 121 °C for 2 h. The partially methylated derivatives were reduced with $NaBH_4$ and acetylated with acetic anhydride and 1-methylimidazole at room temperature for 30 min. Once the reaction was finished, the partially *O*-methylated alditol acetates were extracted with chloroform, dried with $MgSO_4$ and the solvent was evaporated under nitrogen stream.

2.5. Gas chromatography analysis

The partially methylated alditol acetates were separated on a Clarus 500 (Perkin Elmer, Massachusetts, USA) gas chromatograph equipped with a capillary column (Elite 5MS; 60 m × 0.25 mm; 0.25 μm; Perkin Elmer, Massachusetts, USA). Helium was employed as a carrier gas with a flow of 1 mL/min in splitless mode. The injector temperature was maintained at 280 °C. The oven temperature was programmed as follows: held at 80 °C for 10 min, to 150 °C at a rate of 8 °C/min; 150–250 °C at a rate of 3 °C/min, and held for 20 min. Identification and quantification of the partially methylated alditol acetates was achieved by a mass spectrometer (Clarus 560S, Perkin Elmer, Massachusetts, USA) coupled to the gas chromatograph. The mass spectrometer operated at an ion source

temperature of 200 °C and an ionization potential of 70 eV. The analyses were performed on triplicate and the standard deviation was in all cases lower than 1%.

2.6. NMR analysis of polysaccharide fractions

All NMR experiments were performed at Salamanca University (Spain) on a Bruker Avance 400 spectrometer (Bruker BioSciences Española S.A., Madrid, Spain) resonating at 400 MHz for H and at 100 MHz for C. The samples were dissolved in D_2O (Panreac) at a concentration of 10 mg/mL. All spectra were recorded at room temperature (300 K). Chemical shifts were referenced internally to D_2O and DMSO-d6 (4.70 and 2.39 ppm, respectively) for ^1H, and to sodium-3-trimethylsilylpropionate (39.51 ppm) for ^{13}C.

2.7. Colorimetric determination of triple helix with Congo red

The conformational structure of the polysaccharides was established by helix–coil transition analysis according to Ogawa procedure (Ogawa, Tsurugi, & Watanabe, 1972) optimized for fungal polysaccharides (Palacios et al., 2012). Briefly, polysaccharides were dissolved at 500 μg/mL in NaOH solutions (0.05–2 M) and the samples were added to the Congo red solution (40 μM). Spectra were recorded at room temperature (25 °C) on a microplate reader FluoStar Omega (BMG Labtech, Ortenberg, Germany).

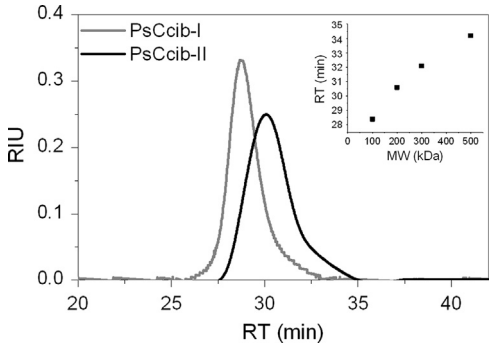

Fig. 1 – HPLC-SEC chromatograms of hot and hot aqueous NaOH fractions (PsCcib-I and PsCcib-II, respectively) isolated from the fruiting bodies of *Cantharellus cibarius* mushrooms using a refractive index detector (Results expressed as refractive index units). Inset: calibration curve with dextran standards.

3. Results and discussion

Three subsequent extractions were carried out for the separation of polysaccharides from the fruiting bodies of *C. cibarius* mushrooms. The first extraction (water at room temperature) was discarded since the yield and purity were very low. Differently, the extraction yield were 1.1% and 9.1% for the hot aqueous (PsCcib-I) and hot NaOH (PsCcib-II) fractions, respectively. Sugar contents were 89.9% and 93.4% in PsCcib-I and PsCcib-II, respectively, whereas protein content was below 1% in both fractions. The purity and the molecular weight of the polysaccharides occurring in each fraction were checked by size exclusion chromatography (Fig. 1). Polysaccharides were diluted in water at 1 g/L. The chromatograms from the two separated extractions from the fruiting bodies of *C. cibarius* mushrooms showed a unique peak in each fraction. The calibration curve prepared with standard dextrans is shown in the inset of Fig. 1. The corresponding molecular weights of the two polysaccharides isolated were approximately 150 kDa (hot fraction, PsCcib-I), and 120 kDa (hot aqueous NaOH fraction, PsCcib-II).

The analysis of the chain composition by GC–MS after acid hydrolysis revealed that both fractions, the hot aqueous polysaccharide (PsCcib-I) and the hot aqueous NaOH polysaccharide (PsCcib-II), were composed of glucose, which indicated the presence of glucan-like polysaccharides.

The analysis by GC–MS of the *O*-permethylated polymers revealed the presence of 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucose; 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucose; and 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylglucose in the hot fraction (PsCcib-I). Differently, the hot aqueous NaOH fraction (PsCcib-II) contained 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucose; 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylglucose and 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methylglucose. Both fractions (PsCcib-I and PsCcib-II) also contained the partially methylated alditol 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucose ascribed to terminal glucose residues (Table 1). The calculated molar ratios between the partially methylated alditols suggested that the hot fraction consisted mainly in (1→6)-linked polysaccharide with (1→4)-linked side chains. Differently, the hot aqueous NaOH fraction contained a polysaccharide composed of (1→3)-linked glucose units with

Table 1 – GC–MS data arising from the methylation analyses of the two polysaccharides (PsCcib-I and PsCcib-II) isolated from the fruiting bodies of the mushroom *Cantharellus cibarius*.

Fraction	Methylated sugar	Major MS fragments	% Area	Linked-glucose
PsCcib-I	2,3,4,6-Me_4-Glc*p*	101, 117, 145, 161	22.5	Glc*p*-(1→
	2,3,4-Me_3-Glc*p*	101, 117, 129, 161	40.2	→6)-Glc*p*-(1→
	2,3,6-Me_3-Glc*p*	101, 113, 117, 161	21.4	→4)-Glc*p*-(1→
	2,3-Me_2-Glc*p*	87, 101, 117, 129	15.8	→4,6)-Glc*p*-(1→
PsCcib-II	2,3,4,6-Me_4-Glc*p*	101, 117, 145, 161	20.6	Glc*p*-(1→
	2,3,4-Me_3-Glc*p*	101, 117, 129, 161	17.2	→6)-Glc*p*-(1→
	2,4,6-Me_3-Glc*p*	101, 117, 129, 161	41.6	→3)-Glc*p*-(1→
	2,4-Me_2-Glc*p*	87, 101, 117, 129	20.5	→3,6)-Glc*p*-(1→

branches at O-6. The degree of branching (DB) was 35% for PsCcib-I and higher than 30% for PsCcib-II, according to the molar ratios of branched to linear glucose residues.

In order to get more insight into the chemical structure of the two polysaccharides extracted from the mushroom *C. cibarius*, a detailed NMR study on the carbohydrates was performed. Fig. 2 shows the ^{1}H NMR spectra for the hot aqueous polysaccharide (PsCcib-I) and the hot aqueous NaOH polysaccharide (PsCcib-II), and Table 2 reviews the peak assignments. The general representative peaks of carbohydrate were ascribed as follows: anomeric proton (H1) signals of glycosides assigned to 4.5–5.0 ppm, and H2, H3, H4, H5 and H6 from glycosidic ring to 3.2–4.5 ppm. The signal for residual D_2O in the sample occurred at the anomeric region in the spectrum at 4.7 ppm. All the signals were assigned based on literature values for similar polysaccharides (Chandra, Ghosh, Ojha, & Islam, 2009; Mandal et al., 2011, 2012; Xu, Xu, & Zhang, 2012).

Two anomeric protons were found in the spectrum from the hot fraction (PsCcib-I), corresponding to the residues **A** and **B**. The peak at 4.88 ppm in addition to the ^{13}C NMR signal at 101.8 ppm suggested that this residue displayed α-type glycosidic bonds. In contrast, the ^{1}H and ^{13}C NMR signals found at 4.50 and 105.0 ppm, respectively, indicated that the other linkage within the structure was β-type. The ^{1}H NMR spectrum of the hot NaOH aqueous fraction (PsCcib-II) showed apparently only one high field anomeric signal at 4.48 ppm, which could be ascribed to the β-linked glucopyranosyl residues.

The 2D NMR spectra of the polysaccharides confirmed the peak assignments and revealed other structural data. The cross-peaks of protons and carbons of each of the sugar moieties were examined in the HMQC spectra (Figs. 3 and 4). The peaks corresponding to the (1→4,6)-linked glucopyranosyl residues from PsCcib-I are not shown in Fig. 3 because they were overlapped to the other signals. Similarly, the signals corresponding to the (1→3,6)-linked glucose from PsCcib-II were very low and overlapped to the other peaks,

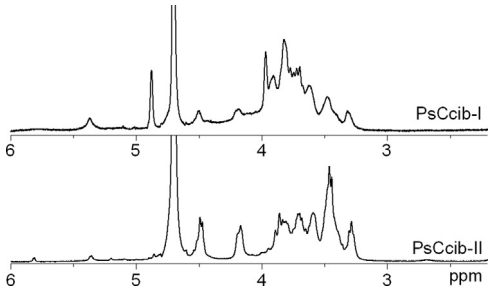

Fig. 2 – ^{1}H NMR spectra of the polysaccharides isolated from the two fractions (PsCcib-I and PsCcib-II) from the fruiting bodies of *Cantharellus cibarius* mushrooms recorded in D_2O at room temperature.

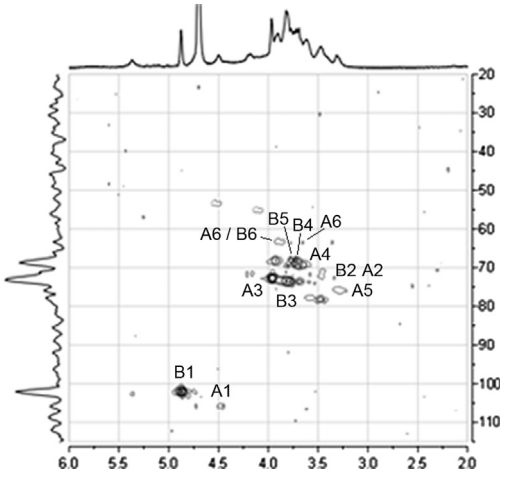

Fig. 3 – ^{1}H–^{13}C HMQC spectrum of the polysaccharide PsCcib-I isolated from *Cantharellus cibarius* mushrooms recorded in D_2O at room temperature.

Table 2 – ^{1}H and ^{13}C NMR spectral assignments of the two polysaccharides (PsCcib-I and PsCcib-II) isolated from the fruiting bodies of the mushroom *Cantharellus cibarius*.

Fraction	Linked-glucose	Chemical shifts (δ, ppm)					
		H1	H2	H3	H4	H5	H6
		C1	C2	C3	C4	C5	C6
PsCcib-I	**A**: →4)-β-Glc*p*-(1→	4.50	3.47	3.81	3.47	3.30	3.70/3.91
		105.0	71.8	72.3	78.2	75.4	63.1
	B: →6)-α-Glc*p*-(1→	4.88	3.47	3.71	3.61	3.80	3.91/3.92
		101.8	71.9	72.5	69.9	70.0	68.0
	C: →4,6)-β-Glc*p*-(1→	4.87	3.47	3.80	3.47	3.30	3.91/3.92
		101.9	71.9	72.4	78.2	72.1	68.0
PsCcib-II	**A**: →3)-β-Glc*p*-(1→	4.48	3.28	3.70	3.45	3.58	3.89/3.71
		106.0	78.2	86.9	75.2	78.4	63.2
	B: →6)-β-Glc*p*-(1→	4.68	3.43	3.61	3.44	3.46	4.17/4.19
		105.7	75.1	78.4	71.4	75.8	71.3
	C: →3,6)-β-Glc*p*-(1→	4.48	3.43	3.17	3.44	3.46	4.17/4.19
		105.9	75.0	86.9	71.4	75.8	71.3

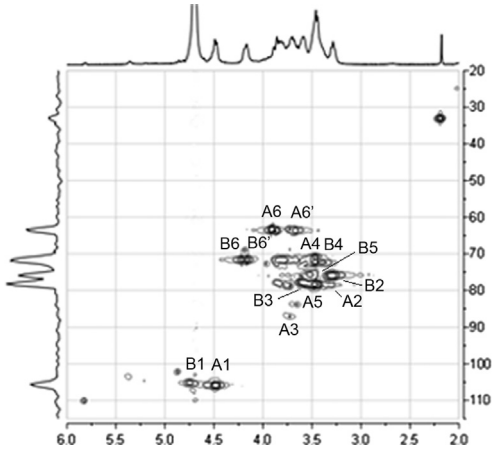

Fig. 4 – ^{1}H–^{13}C HMQC spectrum of the polysaccharide PsCcib-II isolated from *Cantharellus cibarius* mushrooms recorded in D_2O at room temperature.

therefore, they are not shown in Fig. 4. The chemical shifts are reviewed in Table 2. Anomeric carbon (C1) signals of glycosides were assigned at 100–104 ppm. The carbons C2, C3, C4 and C5 from glycosidic ring resonated between 80 and 70 ppm whereas C6 appeared at 63–71 ppm.

In the spectrum of PsCcib-I, the downfield shift of carbon signal of δ 78.2 (C4, residue **A**) from the chemical shift of standard glucose (data not shown) indicated that the residue **A** was (1→4)-linked (Mandal et al., 2011). Similarly, the low field carbon signal at 68.0 ppm (C6 from residues **B** and **C**) indicated that the residues **B** and **C** were (1→6)-linked glucopyranoses (Mandal et al., 2010, 2012). The signals observed in the HMQC spectrum of PsCcib-I agreed with the methylation analysis and the assigned ^{1}H NMR signals. A possible structure for the repeating unit of the branched (1→6),(1→4)-linked glucan is shown below:

→6)-α-D-Glc-(1→6)-α-D-Glc-(1→6)-α-D-Glc-(1→
4
↑
1
[β-D-Glc]$_2$

The presence of (1→3) linkages within the polysaccharide PsCcib-II was confirmed by the downfield shift carbon signal of δ 86.9 (C3 from residue **A**) from the chemical shift of standard glucose. The HMQC spectrum of PsCcib-II (Fig. 4) revealed the presence of an additional anomeric proton at 4.68 ppm (C1 from residue **B**), overlapped with the water signal, and cross-linked to a carbon peak at 105.6 ppm. According to the low chemical shifts and the methylation analysis results, this peak could be identified as the anomeric atoms of β-linked glucopyranose from (1→6)-linked residues (**B**). The low field signal of C6 from residue **B** at 71.3 ppm established that these units were (1→6)-linked glucopyranoses, which confirmed the results of the methylation analysis. The obtained NMR signals for PsCcib-II are attributable to a β-(1→3)-D-glucan with a possible repeating unit structure as shown below:

→3)-β-D-Glc-(1→3)-β-D-Glc-(1→3)-β-D-Glc-(1→
6
↑
1
[β-D-Glc]$_2$

Polysaccharides existing in an ordered three-dimensional structure, generally triple helical conformation, form a complex with Congo red in dilute NaOH solutions. The Congo red molecule can be entrapped in the hydrophobic cavity of the triple helical polysaccharide, and the complex is stabilized by hydrogen bonds and/or hydrophobic interactions between the polysaccharide and the dye molecule (Ogawa, Dohmaru, & Yui, 1994). The complexation of polysaccharides with Congo red can be evaluated by the shift in the visible absorption maxima (λ_{max}) of the Congo red spectrum. Thus, polysaccharides displaying a triple helix conformation would show a shift in UV absorption spectrum whereas other conformations would not show any shift. Polysaccharides from *C. cibarius* were allowed to complex with Congo red. Laminarin and dextran were used as triple-helical and random coil controls, respectively (data not shown), and Congo red in NaOH was evaluated as negative control. Fig. 5 shows the absorption spectra of Congo red in NaOH and polysaccharides complexes with Congo red. Hot fraction (PsCcib-I) did not show any spectral shift after reaction with Congo red, which could indicate that PsCcib-I arranged into a single helical structure. In contrast, the NaOH fraction (PsCcib-II) presented a 10 nm bathochromic shift, which suggested that the polysaccharide formed a complex with the dye. Several studies have shown that the β-glucan mediated immunopharmacological activity may be dependent on the helical conformation, being the triple helix generally more active than the single helix conformer (Ohno, Noriko, Miura, Chiba, Adachi, & Yadomae, 1995). Therefore, the bathochromic shift observed in the NaOH aqueous polysaccharide (PsCcib-II), presenting β-type linkages, could be ascribed to the arrangement into triple strand helical chains in water and the biological activity of this macromolecule could be expected.

This article describes, apparently for the first time, the chemical structure of the polysaccharides isolated from the fruiting bodies of the mushroom *C. cibarius*. The structural pattern consisting of an α-(1→6)-linked chain with β-(1→4) branches (fraction PsCcib-I) has been scarcely described. A similar moiety was found in *L. edodes* mushrooms, however, the linkages were all β-type (Lee, Lee, Cho, Kim, & Hong, 2009). The occurrence of α-(1→4,6) linkages is common in nature, and these polysaccharides usually act as energy storage molecules (Larsen, Essen-Gustavsson, Jensen-Waern, Lametsch, Karlsson, & Lindahl, 2011); nevertheless, the introduction of β-type linkages within the structure may provide different arrangement properties and, therefore, other biological functions. The presence of both linkage types (α and β) within the same macrostructure has been previously described in several polysaccharides obtained from different mushroom species, such as *Pleurotus florida* (Santos-Neves et al., 2008), *Calocybe indica* (Mandal et al., 2010), *Termitomyces microcarpus* (Chandra et al., 2007), or *Auricularia polytricha* (Song & Du, 2012), among others.

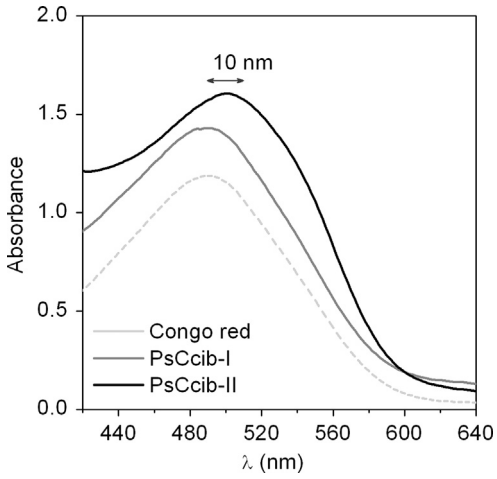

Fig. 5 – Absorption spectra of Congo red (control), and Congo red with the polysaccharides isolated from the hot aqueous and aqueous NaOH fractions (PsCcib-I and PsCcib-II, respectively) from *Cantharellus cibarius* mushrooms.

Regarding the PsCcib-II fraction, the β-(1→3),(1→6)-linked polysaccharides so far isolated from mushrooms have essentially similar structural features, although there are differences in the size and shape, the distribution, length and the degree of branching, the three dimensional arrangement, etc. The moiety β-(1→3),(1→6) has been previously found in different genera, such as *Lentinus* (Xu, Chen, Zhang, & Ashida, 2012), *Pleurotus* (Rout, Mondal, Chakraborty, & Islam, 2008), *Hericium* (Dong, Jia, & Fang, 2006), etc.; and this structural pattern has demonstrated to be responsible of the beneficial properties of β-glucans including the promotion of healthy cholesterol and blood glucose levels and the enhancement of the immune system functions (Sze & Chan, 2012; Xu et al., 2012).

The structure of the polysaccharide has shown to highly influence the biological activity in terms of solubility, molecular interactions and bioavailability (Surenjav, Zhang, Xu, Zhang, & Zeng, 2006; Yoshioka, Uehara, & Saito, 1992); therefore, the study of the chemical arrangement of natural polysaccharides gives more insight into the beneficial properties of mushrooms and their components.

4. Conclusion

The isolated carbohydrates from *C. cibarius* were glucan-type polysaccharides composed of glucose. Two main fractions were obtained by hot water and hot aqueous NaOH extractions, respectively. The polysaccharide from hot fraction (PsCcib-I) was composed of a main chain of α-(1→6)-linked glucopyranosyl residues with β-(1→4)-linked branches. Differently, the polysaccharide from hot aqueous NaOH fraction (PsCcib-II) was formed by β-(1→3)-linked glucose units with branches at O-6. These results suggest that different kinds of glucans can be obtained from other edible mushrooms, for instance *C. cibarius*, although the pattern of binding and branches depended on the extraction procedure.

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