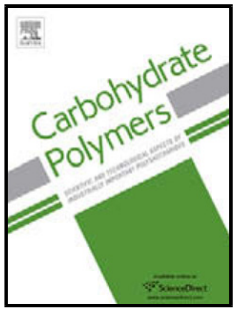

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Structural characterization and rheological properties of a gel-like β-D-glucan from *Pholiota nameko*

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Highlights

- β-D-glucan fractions were isolated from fruiting bodies of *Pholiota nameko*;
- NMR and methylation analyses confirmed the presence of (1→3),(1→6)-β-D-glucan;
- Crude and purified β-D-glucan showed shear-thinning behavior;
- The tested fractions presented thermal stability in a wide temperature range;
- *P. nameko* glucans are promising thickener and gelling agents for food products.

Abstract

A crude fraction (SCW) was extracted with cold water from *Pholiota nameko* and showed mannose (24.1%), galactose (44.9%) and glucose (31%). Purification procedures resulted in a polysaccharide fraction (bG-PN), that showed only glucose. NMR and methylation analyses of bG-PN indicated a β-D-glucan-(1→3)-linked, substituted at O-6 by β-D-Glc*p* or (1→6)-linked β-D-Glc*p* side chains. Rheological studies of crude and purified fractions at the same concentration showed similar shear-thinning behavior and gel-like structure which indicates no need to isolate the polymer to achieve some desirable rheological properties. SCW (at 1% and 2%) and bG-PN (at 2%) presented thermal stability during heating and cooling, suggesting that the physical structure of gels (SCW and bG-PN at 2%) and viscoelastic fluid (SCW at 1%) formed were not altered in the tested temperature range. Our

results suggest that *P. nameko* β-D-glucans can be applied in different food preparations as thickener or gelling agents modifying their rheological properties.

Keywords: *Pholiota nameko,* structural characterization, β-D-glucan, rheology.

1. Introduction

Edible mushrooms have been considered for many years as functional foods due to their high nutritional value (Ren, Perera, & Hemar, 2012). They present an attractive chemical composition, being rich in proteins, carbohydrates, fibers, vitamins and unsaturated fatty acids (Wani, Bodha, & Wani, 2010). Mushrooms have showed a great interest in biomedical area due to the presence of bioactive substances, including polysaccharides (Zhao, Wang, & Lu, 2009). *Pholiota nameko* is a well known edible mushroom rich in biological compounds, being extensively cultivated to be used as food and as traditional medicine in China and Japan (Neda, 2008; Zhang et al., 2014).

Studies showed that *P. nameko* polysaccharides demonstrated strong anti-inflammatory activity in rodents through paw edema model (Li, Lu, Zhang, Lu, & Liu, 2008) and hypolipidemic effect after orally treatment in hyperlipidemic rats (Li, Zhang, & Ma, 2010). Furthermore, this mushroom can be useful for food industry to design supplements due to its potential as prebiotic (Rodrigues et al., 2017). Fungal polysaccharides comprise a large group of polymers with pharmaceutical and biotechnological applications and such properties are closely related to their chemical structures (Bot et al., 2001; Ruthes, Smiderle, & Iacomini, 2015). The most studied class of polysaccharides present in edible mushrooms are the β-D-glucans which frequently consist of a backbone composed of β-(1→3)-linked glucopyranose, partially substituted at O-6 position by glucopyranose units (Ren, Reynisson, Perera, & Hemar, 2013; Synytsya & Novak, 2014).

Mushroom nutritional values associated with their therapeutic benefits stimulate the application of such organisms or their extracts in food industry as additives, supplements and emulsifiers (Liu et al., 2016; Xu, Xu, Zhang, & Zhang, 2008; Xu, Zhang, Liu, Sun, & Wang, 2016). β-D-Glucans can present special physicochemical properties such as gelling capability, which might be employed in food, pharmaceutical and cosmetic industry (Laroche & Michaud, 2007). The determination of their rheological characteristics is helpful to predict gelation, thickening and emulsification properties. β-D-Glucans are abundant polysaccharides in edible mushrooms and have potential to increase water viscosity and provide desirable appearance and texture for food products (Bot, Smorenburg, Vreeker, Pâques, & Clark, 2001; Synytsya & Novak, 2014). The viscosity promoted by such molecules in solution is related to their molecular weight, chemical structure, concentration and food matrix (Zhu, Bin, Bian, & Xu, 2015).

Few reports have mentioned some food industry applications using β-glucan, such as rice noodles prepared with β-glucan-rich fractions from *L. edodes* (Heo et al., 2013); and wheat flour mixture containing β-glucan-enriched material from the same mushroom to prepare cakes (Kim et al., 2011). In both cases, the addition of mushroom β-glucans enhanced the quality of the products by improving texture characteristics and increasing the fiber content.

Observing the potential use of mushroom β-glucan-rich fractions for food industry, this study aimed to isolate, purify and structurally characterize a gel-like β-D-glucan from *Pholiota nameko*, including studies on rheological properties of the crude and purified fractions.

2. Material and Methods

2.1 Fungal material

Pholiota nameko was kindly provided by the company Nayumi Cogumelos, located in the city of Mogi das Cruzes, São Paulo, Brazil.

2.2 Extraction and purification

Pholiota nameko (PN) (2.5 kg) was freeze dried until total dryness (226.0 g) and milled in a blender (Fig. 1). The apolar compounds were extracted using a chloroform-methanol solution (2:1 v/v) at 60 °C for 5 h (3 times). Subsequently, the residue was dried and submitted to sequential aqueous extractions at room temperature (23 °C) under mechanical stirring for 6 h (3 times). The aqueous extract was concentrated under reduced pressure and the polysaccharide was precipitated with ethanol (3:1 v/v). The ethanolic solution was centrifuged at 8,500 rpm at 10 °C for 20 min and the precipitate was dialyzed (12-14 kDa M_r cut-off membrane) against tap water for 24 hours and freeze dried.

The resultant fraction (CW; 37.4 g) was submitted to freeze-thawing process (Gorin & Iacomini, 1984). This method consists of solubilizing the sample in small volume of distilled water followed by freeze and slowly thawing at room temperature (23 °C) until the formation of precipitates. The soluble fraction (SCW; 17.9 g) was separated from the insoluble one by centrifugation (8,500 rpm, 4 °C, 20 min) and treated with Fehling solution (Jones & Stoodley, 1965). The fraction (FSCW; 1.2 g) which did not complex with Cu^{2+} ions was recovered after centrifugation (8,500 rpm, 4°C, 20 min) and separated from the insoluble Cu^{2+} complex, which was not used in this study.

The obtained fractions were neutralized with HOAc, dialyzed against tap water for 48 hours (12-14 kDa M_r cut-off membrane) and deionized on a cationic resin ion exchange and then freeze dried. The FSCW fraction was submitted to another freeze–thawing process and centrifuged (8,500 rpm, 4°C, 20 min): resulting in a soluble fraction (not used in this study) and a gel fraction (bG-PN; 0.52 g), that was used for the chemical and rheological analysis.

2.3 Analysis of monosaccharide composition by GC-MS

Aliquots of polysaccharide fractions (1.0 mg) were hydrolyzed with 2 M TFA for 8 hours at 100 °C following the method of Sassaki et al. (2008). The acid was removed by evaporation to dryness. The hydrolyzed products were dissolved in distilled water (100 µL) and 1 mg of $NaBH_4$ was added. The solution was held overnight in room temperature (23 °C) to reduce aldoses into alditols, neutralized with HOAc and later evaporated to dryness. The residue was removed following the addition of methanol (2 times) under a compressed air stream in a fume hood. The samples were acetylated with pyridine–Ac_2O (200 µL; 1:1, v/v), heated for 30 min at 100 °C. The resulting alditol acetates were extracted with chloroform and analyzed by gas chromatography-mass spectrometry (GC-MS) using a Varian model CP-3800 gas chromatograph coupled to an Ion-Trap 4000 mass spectrometer, with a VF5 column (30 m x 0.25 mm i.d.) programmed from 100 to 280 °C at 10 °C/3 min, with He as carrier gas. The alditol acetates were identified by their typical retention times and electron impact profiles.

2.4 Methylation analysis

Per-*O*-methylation of purified polysaccharide (bG-PN) fraction was carried out using NaOH–Me_2SO–MeI according to Ciucanu and Kerek (1984). After isolation of the products by neutralization (HOAc), dialysis, and evaporation, the methylation process was repeated. The per-*O*-methylated products were submitted to methanolysis with MeOH-HCl 3 N (1 mL) for 2 h at 80 °C (Jannson, Kenne, Liedgren, Lindberg, & Lonngren, 1976) followed by hydrolysis using 2 N sulphuric acid (1 mL) for 16 h at 100 °C. The sample was reduced with $NaBD_4$ and acetylated to give a mixture of partially *O*-methylated alditol acetates, which was analyzed by GC-MS. Partially *O*-methylated alditol acetates were identified from m/z by comparing their positive ions with standards and expressed as a relative percentage of each component.

2.5 Controlled Smith degradation of β-D-glucan (bG-PN)

The purified fraction (bG-PN; 50 mg) was oxidized with 0.05 M aqueous $NaIO_4$ (20 mL) at room temperature (23 °C) protected from light for 72 hours. Ethylene glycol was added to stop the reaction, the material was dialyzed (2 kDa M_r cut-off membrane) and the resulting polyaldehyde was reduced with $NaBH_4$ for 12 hours, neutralized with HOAc, dialyzed and concentrated to 50 mL (Goldstein, Hay, Lewis, & Smith, 2005). The residue was submitted to partial hydrolysis with TFA, pH 2.0, for 30 minutes at 100 °C and dialysis (2 kDa M_r cut-off membrane). The final solution was freeze dried the material resistant to oxidation was analyzed by ^{13}C-NMR spectroscopy.

2.6 Nuclear Magnetic Resonance

^{13}C- and HSQC-NMR spectra were obtained using a 400 MHz Bruker model Avance III spectrometer with a 5 mm inverse probe. The analyses were performed at 70 °C and polysaccharide samples (40 mg) were dissolved in Me_2SO-d_6. Chemical shifts are expressed in ppm (δ) relative to Me_2SO-d_6 ^{13}C and ^{1}H signals at δ 39.40 and 2.40, respectively.

2.7 Rheological measurements

Rheological analysis were made on crude fraction SCW (at 1% and 2% w/w), that presented 48.4% yield and on the isolated β-D-glucan fraction (bG-PN), at 2% (w/w) due its low yield (1.4%). Samples were solubilized in distilled water being maintained under magnetic stirring for 5 days at 70 °C and rest during 20 minutes before analyses. Rheological measurements were carried out using a HAAKE MARS II rheometer (Thermo Fisher Scientific, Karlsruhe, Germany), at 25 °C with a cone-plate (C60/2° TiL) measurement system. A 1 mm measurement gap was used. The temperature was controlled by a circulating water bath (DC5, Haake) coupled to a Peltier temperature control device (TC 81, Haake). During measurements on temperature variation, the system containing sample was covered with a sample hood (POM 222-1903) to prevent water evaporation. Before all rheological measurements, samples were maintained on the plate for 300 s, in order to allow temperature equilibrium. Viscosity curves were obtained in the CR mode (controlled shear rate) by applying an increasing shear rate (0.005 to 500 s^{-1}) during 300 s. Viscoelastic behavior of samples were evaluated using frequency sweeps (0.02 to 10 Hz) with strain of 1%. On thermostability studies, temperature sweeps were performed by heating (5 to 60 °C) and subsequent cooling (60 to 5 °C) the samples at a rate of 2 °C/min, at a frequency of 1 Hz and strain of 1%. The software RheoWin 4 Data Manager was employed to obtain the rheological parameters. All the analyses were performed in triplicates and graphs show the mean values and their corresponding standard error of the mean (SEM).

3. Results and Discussion

3.1 Preparation of polysaccharide extracts and isolation of β-D-glucan

Polysaccharide fractions were obtained from *Pholiota nameko* fruiting bodies after successive cold water extractions as explained above (section 2.2). Table 1 presents the monosaccharide composition of each fraction obtained over the purification process.

All fractions analyzed presented mannose, galactose and glucose, being this last monosaccharide the most abundant in CW (79.6%) and bG-PN (100%) fractions. The composition observed for CW and SCW indicated the presence of a mixture containing a D-glucan and an heteropolysaccharide composed of mannose and galactose as observed in

previous reports that isolated mannogalactans from basidiomycetes (Smiderle et al., 2008; Silveira et al., 2015). Fehling solution treatment was effective to separate the D-glucan from the other heteropolysaccharide, as confirmed by the monosaccharide composition of bG-PN (Table 1).

3.2 Chemical composition of SCW fraction and characterization of β-D-glucan

The SCW fraction presented a mixture of monosaccharide components (Table 1) and was submitted to treatment with Fehling solution and freeze-thawing purification process giving rise to bG-PN fraction. NMR spectra of crude and purified fractions are shown in Fig. 2. ^{13}C-NMR spectrum of the crude extract SCW (Fig. 2A) indicated a mixture of polysaccharides due to the presence of various anomeric signals.

The main C-1 signals that correspond to β-D-Glc*p* (δ 102.8), β-D-Man*p* (δ 101.5), α-D-Glc*p* (δ 100.2) and α-D-Gal*p* (δ 98.8) were identified by comparison with previous reports (Rosado et al., 2003; Smiderle et al., 2006; Smiderle et al., 2010). The signal at δ 79.1 arises from O-4 substitution of α-D-Glc*p* residues characteristic of glycogen-like polysaccharides, which are normally encountered in mushrooms (Smiderle et al., 2010; Stanek, Falk, Huber, 1998). The presence of mannogalactans in SCW fraction was confirmed by the signals at δ 76.8 and δ 66.9, corresponding to O-2 and O-6 substitutions of α-D-Gal*p* residues (Silveira et al., 2015). Furthermore, signals at δ 85.8; 86.0; and 86.4 indicated O-3 substitution of β-D-Glc*p* residues (Moreno et al., 2016).

Methylation analysis of bG-PN showed the presence of 2,3,4,6-Me_4-Glc*p* (27%); 2,4,6-Me_3-Glc*p* (30%); 2,3,4-Me_3-Glc*p* (16%); and 2,4-Me_2-Glc*p* (27%) derivatives. These data indicate that bG-PN presents a backbone composed of Glc*p*-(1→3)-linked, highly substituted at O-6 by Glc*p* residues and/or side chains of Glc*p*-(1→6)-linked. A similar D-glucan, with the same proportion of side-branches was isolated previously from *Cookeina tricholoma* by alkaline extraction (Moreno et al. 2016). Interestingly, in the present study, the D-glucan was easily extracted by water.

To confirm methylation results, bG-PN was analyzed by NMR spectroscopy (Figs. 2B and 3).

The ^{13}C-NMR spectrum contained only one anomeric signal at δ 102.8 (Fig. 2B), while it was observed two distinct signals (C-1/H-1) in HSQC experiment, one at δ 102.4/4.12 corresponding to non-reducing end-units, and another at δ 102.4/4.41 from 3-*O*- and 3,6-di-*O*-substituted residues. The β-configuration was confirmed by low frequency H-1 (δ 4.12 and 4.41) and high-frequency C-1 signals (δ 102.4) (Hall & Johnson, 1969). The

glycosidic linkages suggested by the methylation analyses were confirmed by the presence of 3-*O*-substitution signals at δ 86.5, 86.1 and 85.8 (Figs. 2B and 3) and 6-*O*- substitution signals at δ 68.3 (Fig. 2B), which appeared as doublet in HSQC spectrum at δ 68.1; 3.96/3.41 (Fig. 3) and was confirmed by RMN-^{13}C-DEPT experiment (data not shown). Non-substituted C-6 signals were also observed and are assigned at δ 60.7 (Fig. 2B), 60.5/3.58 and 60.6/3.36 (Fig. 3) (Carbonero et al., 2012). The other signals in HSQC spectrum at δ 72.3/3.20; 68.1/3.15; 75.9/3.08 are attributed to C2/H2, C4/H4, and C5/H5 respectively, of β-D-Glc*p*-(1→3)-linked main chain. Similar signals relative to mushroom β-D-glucans were observed by Carbonero et al. (2006), Ruthes et al. (2013) and Moreno et al. (2016).

The main chain of the isolated β-D-glucan (bG-PN) was confirmed after performing a controlled Smith degradation that consists of $NaIO_4$ oxidation followed by mild acid hydrolysis. Only (1→3)-linked residues are resistant and all branching residues were totally oxidized by this procedure and after analyzing the residual product by ^{13}C-NMR, six intense signals were observed (Fig. 4). It proved to be a linear (1→3)-linked β-D-glucan with six typical signals at δ 102.8; 86.1; 76.3; 72.8; 68.3 and 60.7 ppm corresponding to C-1, C-3, C-5, C-2, C-4, and C-6, respectively (Gorin, 1981).

The results obtained by the chemical analyses are in agreement and confirmed the presence of a β-D-glucan with a main chain (1→3)-linked and highly substituted at O-6 by non-reducing β-D-Glc*p* or β-D-Glc*p*-(1→6)-linked side chains.

Similar β-D-glucans from *Pleurotus florida* (Rout, Mondal, Chakraborty, Pramanik, & Islam, 2005), *Pleurotus pulmonarius* (Smiderle et al., 2008) and *Cantharellus cibarius* (Nyman, Aachmann, Rise, Balance, & Samuelsen, 2016) have been studied. Rodrigues et al. (2015) observed evidences of β-glucans, α-glucans and glucan-protein complexes by FTIR-ATR in five species of mushrooms including *P. nameko*. However, the chemical characterization of such molecules had not been reported. Studies on biological activities of crude polysaccharide extracts from genus *Pholiota* are commonly found, although there is a lack of information relating the chemical structure of such polymers with their biological effects or rheological properties (Li, Lu, Zhang, Lu, & Liu, 2008; Li, Zhang, & Ma, 2010). A purified (1→3),(1→6)-β-D-glucan from *P. nameko* fungi has not been reported up to this date.

3.3 Rheological characterization of SCW and β-D-Glucan fractions

High degree of polysaccharide branching promotes higher interaction between water and polysaccharides by hydrogen bonds. Such inter- and intramolecular interactions may also influence solution behavior by increasing viscosity or forming gels (Yalpani & Hall, 1984). Fraction SCW and the highly branched β-D-glucan isolated from *P. nameko* showed gelling appearance, therefore their rheological properties were evaluated.

The flow behavior of SCW fraction (at 1% and 2% w/w) was compared with the behavior of isolated β-D-glucan (at 2% w/w) (Fig. 5).

The apparent viscosity of the first one (SCW) was concentration dependent, as zero shear viscosity value increase when the polymer concentration rises. The viscosity observed for 1% SCW dispersion at a shear rate of 0.01 s^{-1} was 4.1 Pa.s and increased to 49.5 Pa.s at 2% of concentration. It was observed that at higher concentrations there is more intermolecular connections promoting "junction zones", which limit movement and stretching of polymers in solution and, consequently increase viscosity (Freitas et al., 2009). SCW aqueous dispersions showed lower viscosity than those observed for aqueous extract from *Flammulina velutipes* (Du et al., 2016) and *Auricularia auricular-judae* (Bao et al., 2016). These could be related to the composition of the extracts, considering that all of them were composed by galactose, mannose and glucose in different proportions. The crude fraction SCW presented higher amounts of galactose (44.9%) in comparison to *A. auricular-judae* and *F. velutipes* fractions, that contained 5.4% (Bao et al., 2016) and 12% of galactose residues (Yang et al., 2012), respectively.

Both tested concentrations of SCW fraction (1% and 2% w/w) showed shear-thinning behavior, as there was an increase of the shear stress and a pronounced decrease of their viscosities, especially after 0.05 s^{-1}, with the increasing of the shear rate (Fig. 5). This is a common behavior of polysaccharide dispersions, where disordered polysaccharides seem to align or deform along the flow direction, decreasing the dispersion viscosity (Lapasin & Pricl, 1995). Such behavior was identified for other polysaccharides from edible mushrooms as *F. velutipes* (Du et al., 2016), floral mushrooms (Xu, Zhang, Liu, Sun, & Wang, 2016), *A. auricular-judae* (Bao et al., 2016), and *Ganoderma lucidum* (Liu et al., 2016).

The flow and viscosity curves of fraction bG-PN at 2% (Fig. 5) were very similar with those observed for SCW at the same concentration, for all tested shear rates, suggesting that it is not necessary to completely purify the β-D-glucan to obtain the desirable viscosity. This result is interesting for the application in industry, which requires less purification steps to avoid unnecessary expenses. Besides, SCW yield was 34 times higher than bG-PN. The purified β-D-glucan from *P. nameko* formed less viscous aqueous dispersion than those isolated from floral mushrooms (Xu, Zhang, Liu, Sun, & Wang, 2016) and *G. lucidum* (Liu et al., 2016), although all of them showed shear-thinning behaviors. The fraction bG-PN (at 2%) presented viscosity of 3.5 Pa.s at 1 s^{-1} while that from floral mushrooms cultivated in Huangshan Mountain have similar viscosity at half of concentration (Xu, Zhang, Liu, Sun, & Wang, 2016). Initial viscosity of bG-PN (at 2%) at first Newtonian plateau, at 0.01 s^{-1}, was 38.4 Pa.s, while β-D-glucan from *G. lucidum* showed almost 150 Pa.s at the same shear rate (Liu et al, 2016).

β-D-Glucan from *P. nameko* showed a highly branched structure, containing longer side chains in comparison to β-D-glucans from floral mushrooms (Xu, Zhang, Liu, Sun, & Wang, 2016) and *G. lucidum* (Liu et al., 2016), that present only non-reducing end units of Glc*p* as branches. Such chemical characteristics may be responsible for the differences observed on viscosity of the three β-D-glucans.

The viscoelastic behaviors of fraction SCW (at 1% and 2% w/w) and isolated β-D-glucan (at 2% w/w) at 25 °C are shown in Fig. 6.

G' and G" of SCW at 1% were crossed-over at low frequency (< 0.05 Hz) characterizing it as concentrated solution and entangled systems (Clark & Ross-Murphy, 1987). When the concentration reached 2% (w/w), SCW showed a gel-like behavior with G' higher than G", both slightly frequency dependent, in all tested frequency range. The increase of concentration promoted the formation of more entanglements and junction points between the polymer chains resulting in formation of a gel network (Brito, Sierakowski, Reicher, Feitosa, & Paula, 2005). The gel-like behavior was also noticed in aqueous extract from mushroom *A. auricular-judae* (named AP) at 0.5%, 1%, and 2%, and gel strength increased with increment of AP concentration (Bao et al., 2016). Our results showed that bG-PN (2% w/w) has similar gel-like behavior than that from aqueous extract SCW at the same concentration (Fig. 6). The β-D-glucan from *P. nameko* formed lower strength gel than that from *G. lucidum*, as G' and G" values of bG-PN at 2% were comparable with those from *G. lucidum* at 1%, at the same temperature (Liu et al., 2016). In contrast, β-D-glucan isolated from floral mushrooms (Xu, Zhang, Liu, Sun, & Wang, 2016) (at 1%) did not form gel at 25 °C (Xu, Zhang, Liu, Sun, & Wang, 2016).

With the aim of investigating thermal stability of SCW and bG-PN, dispersions were heated (from 5 to 60 °C) and cooled (from 60 to 5 °C) and their viscoelastic moduli were determined. SCW (at 1% and 2%) and bG-PN (at 2%) showed strong thermal stability over the temperature course (Fig. 7). During heating and cooling, G' and G" reached almost the same values as the initial temperatures, suggesting that the temperatures tested did not affect the physical structure of gels and viscoelastic fluids formed by both samples.

At the temperature ramps, bG-PN (2% w/w) showed higher G' and G" values than those observed for SCW fraction at the same concentration (Fig. 7). This behavior was different from that observed at frequency sweeps suggesting that at 5 °C, there were more intra- and intermolecular associations between the isolated β-D-glucan molecules, resulting in more junction zones and consequently in more strength gel than that formed at 25 °C. The β-D-

glucan (bG-PN) gel showed to be thermal stable, without strength loss, during the temperature ramp tested.

Conclusion

Polysaccharide extracts from *P. nameko* were obtained by cold water extractions and a highly branched glucan was purified and chemically characterized. Its backbone was composed of (1→3)-linked β-D-Glc*p* units, being 27% substituted at O-6 by single units of β-D-Glc*p* or (1→6)-linked β-D-Glc*p* side chains. The crude extract (SCW) and purified β-D-glucan (bG-PN) showed similar non-Newtonian shear-thinning behavior, and viscosity of SCW fraction was higher when there was an increase in its concentration. SCW and bG-PN (at 2%) showed gel-like behavior and were thermal stable between the range of 5 to 60 °C. Such characteristics suggested a potential application of *P. nameko* fractions to increase thickness of food products and also to improve resistance of products submitted to different temperatures during its processing, storage and/or transport. The shear-thinning behavior and gel-like structure of SCW and bG-PN fractions at 25 °C were similar at the same concentration which suggested there is no need to purify the β-D-glucan to obtain some desirable rheological properties. The results obtained indicate a potential use of β-D-glucan-rich fractions from *P. nameko* in food industry as thickening agents to improve desirable characteristics of food products associated with other therapeutic benefits that are attributed to β-D-glucans.

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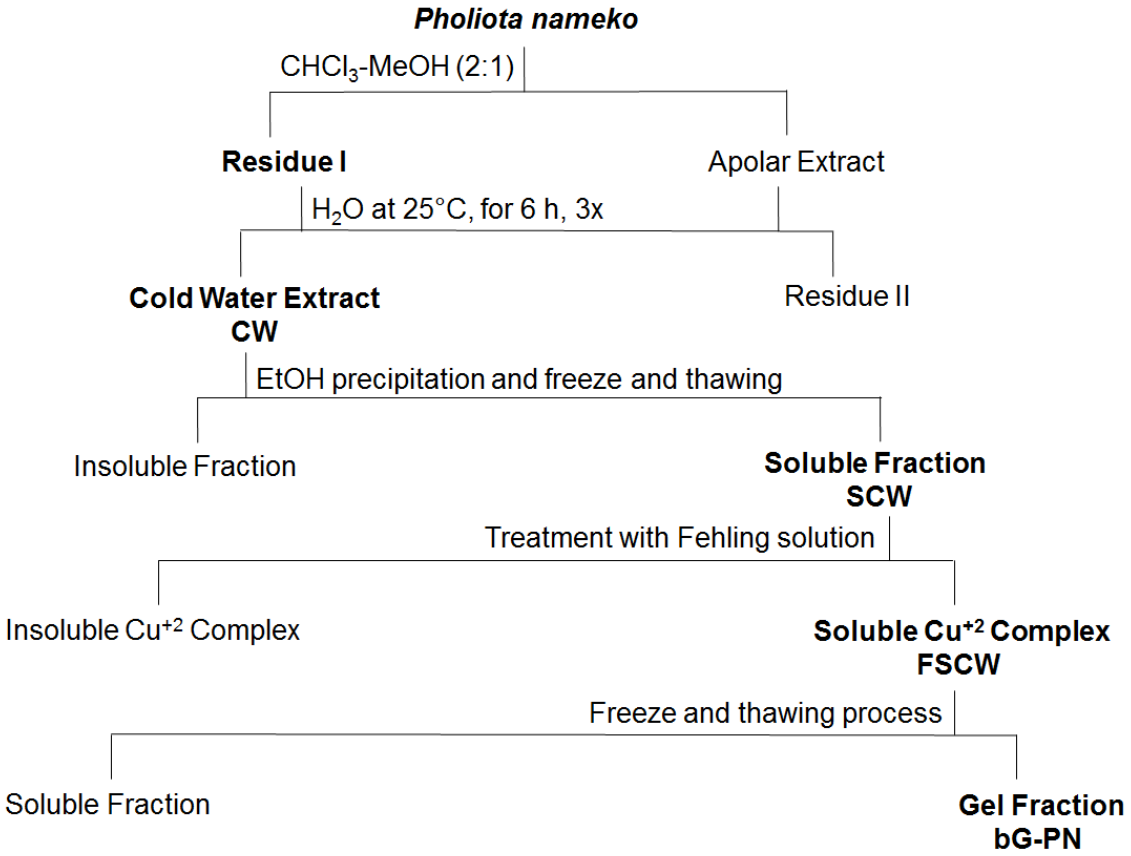

Figure 1: Scheme of extraction and purification of β-D-glucan obtained from fruiting bodies of *Pholiota nameko*.

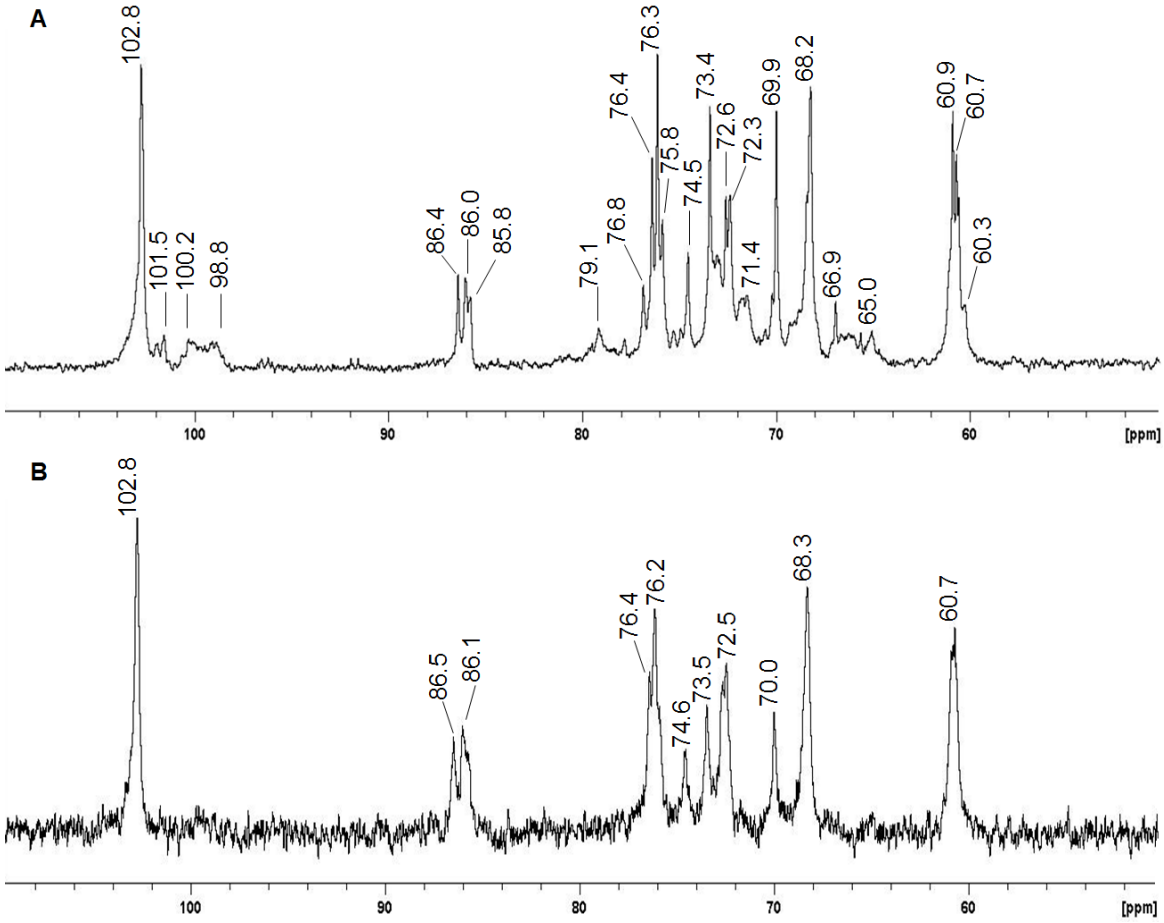

Figure 2: ^{13}C-NMR spectra of SCW (A) and bG-PN (β-D-glucan) (B) fractions in Me_2SO-d_6 at 70 °C (chemical shifts are expressed in δ ppm).

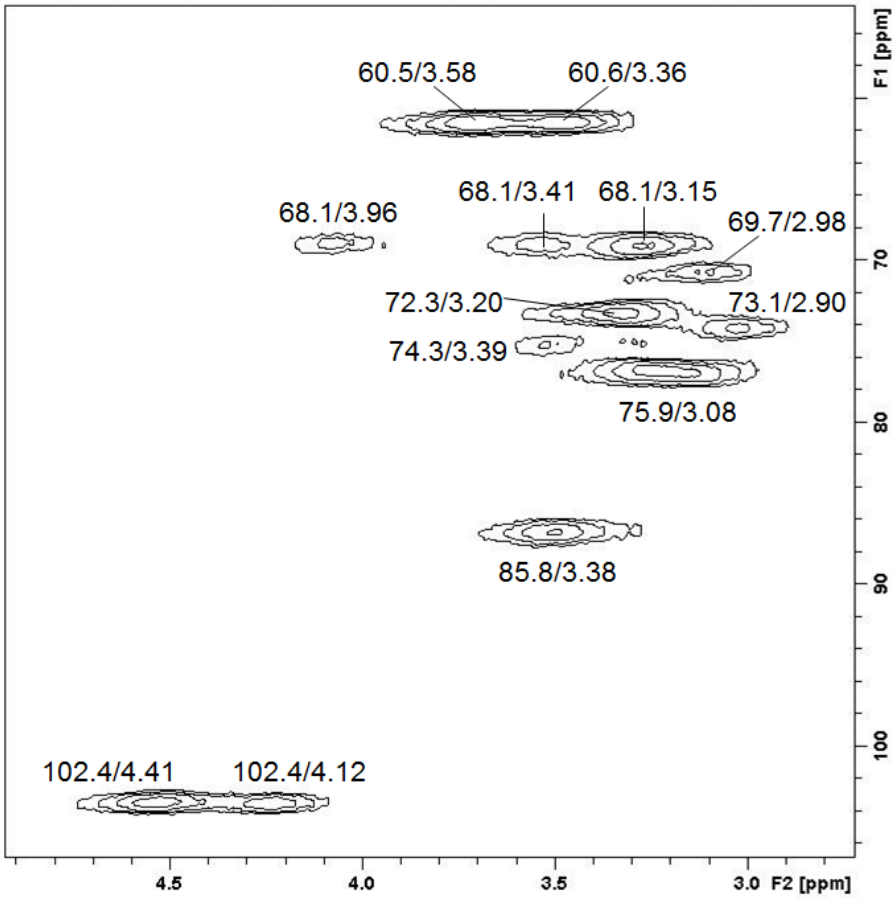

Figure 3: HSQC-NMR spectrum of bG-PN in Me_2SO-d_6 at 70 °C (chemical shifts are expressed in δ ppm).

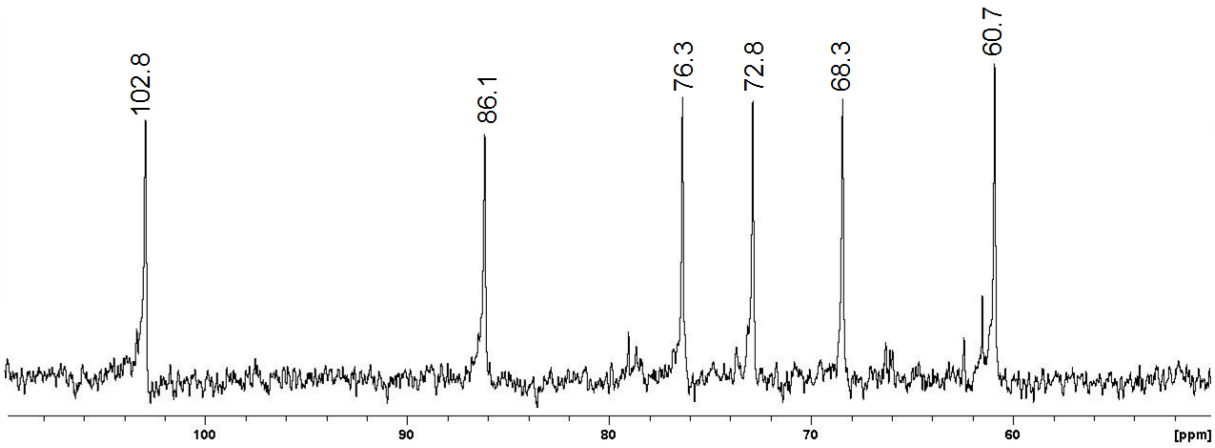

Figure 4: ^{13}C-NMR spectrum of partially degraded glucan from *Pholiota nameko* in Me_2SO-d_6 at 70 °C (chemical shifts are expressed in δ ppm).

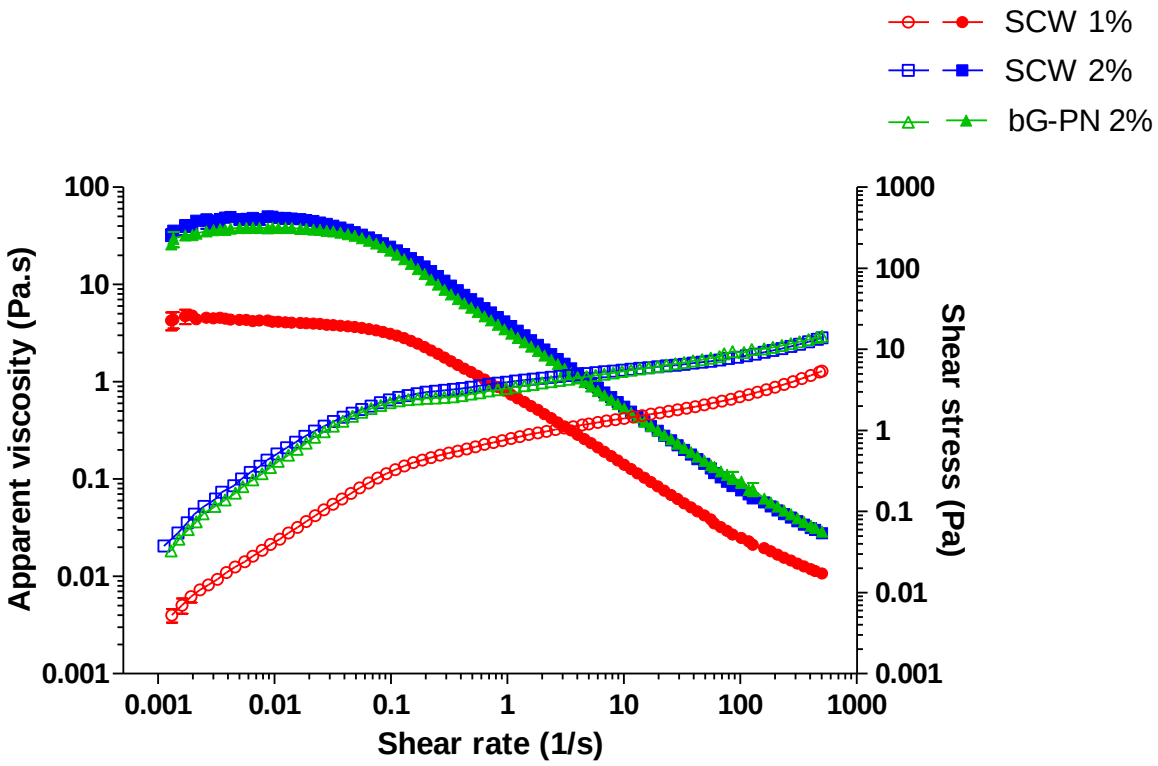

Figure 5: Flow (empty symbols) and viscosity (full symbols) curves of aqueous SCW fraction at 1% and 2% and aqueous bG-PN fraction at 2%.

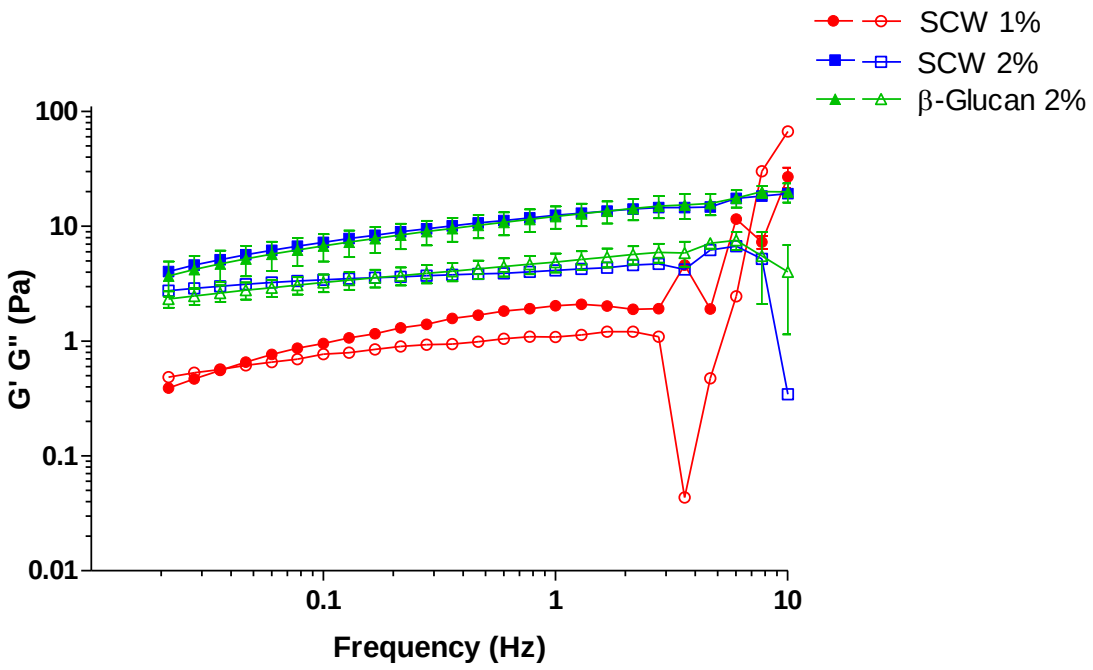

Figure 6: Frequency sweeps at 25 °C of fractions SCW at 1% and 2% and bG-PN at 2%. Elastic modulus (G') is represented by full symbols while viscous modulus (G") by open symbols. Fixed strain of 1%.

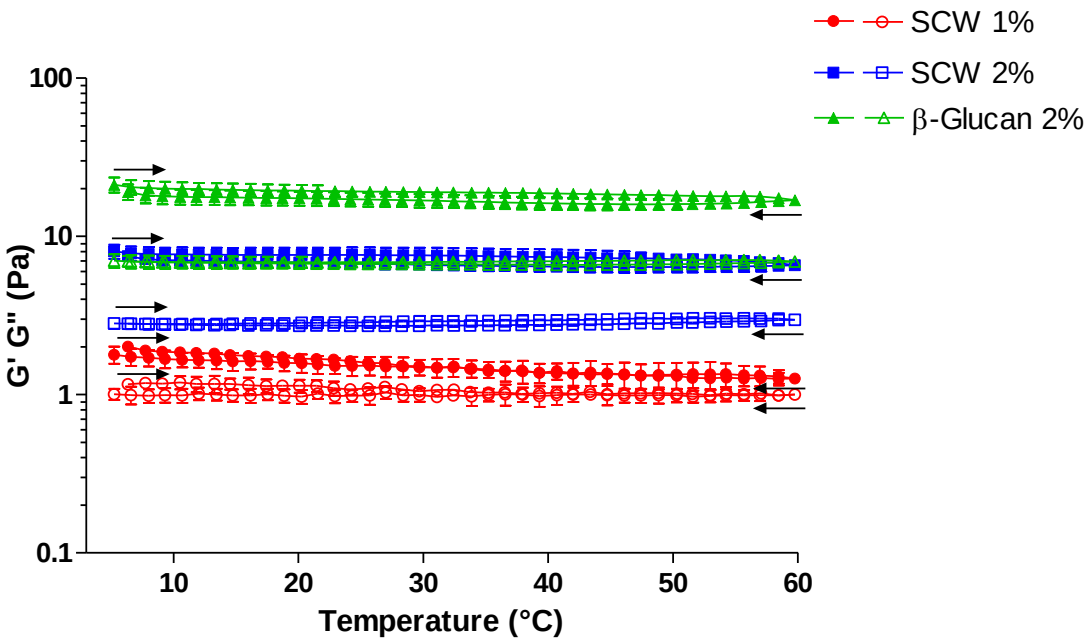

Figure 7: Elastic (G', full symbols) and viscous moduli (G", open symbols) of fractions SCW (at 1% and 2%) and bG-PN (at 2%) as a function of temperature. Fixed frequency at 1 Hz and strain of 1%.

Table 1. Monosaccharide composition of fractions obtained from *Pholiota nameko*.

Fractions	**Monosaccharide Composition (%)[a]**		
	Man	**Gal**	**Glc**
CW	14.1	6.3	79.6
SCW	24.1	44.9	31.0
bG-PN	-	-	100.0

[a] % of peak area relative to total peak areas, determined by GC–MS.