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RESEARCH ARTICLE

Characterization of β-Glucan from Oyster Mushroom (*Pleurotus pulmonarius*)

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ABSTRACT

Mushrooms are known as β-glucan sources. The purpose of this investigation was to characterize and purify β-glucan by alkaline extraction from oyster mushrooms (*Pleurotus pulmonarius*) to evaluate its molecular weight (Mw) and viscosity. The ideal extraction process parameters were found as 80°C, 90 min, and 30% KOH. Fourier transforms-infrared spectroscopy analysis and ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy were used for chemical characterization, and extracted β-glucan was found to be a β-(1-3) glucose polymer with β-(1-6) side chains. The Mw was determined as 349.260 g/mol. Rheological measurements showed that β-glucan has low apparent viscosity in aqueous solutions.

Key words: Fourier transforms-infrared spectroscopy, liquid-chromatography-mass spectrometry, nuclear magnetic resonance, oyster mushroom, viscosity, β-glucan

INTRODUCTION

Over 2000 different kinds of mushrooms may be found in nature, but only nearly 25 of them are commonly consumed as food and just a small number are raised for commercial purposes.[1] Yet, it may be difficult to distinguish between edible and medicinal mushrooms since various of popular food species offer medicinal benefits and numerous medicinal mushrooms are also edible. Eating mushrooms contains a lot of carbohydrates, such as chitin, glycogen, trehalose, and mannitol, as well as fiber, β-glucans, hemicelluloses, and pectic materials. Moreover, produced edible mushrooms contain large levels of the sugars glucose, mannitol, and trehalose, but only modest amounts of fructose and sucrose. They offer exceptional nutritional value since they are low in fat but high in essential fatty acids, relatively rich in protein, and include a high amount of fiber and necessary amino acids.[2]

The food and drug administration has determined that to lower total blood cholesterol levels, and it is suggested to have a daily minimum intake of 3 g of β-glucans. The health statement simply mentions the required amount of oat β-glucans; however, it makes no mention of the physicochemical characteristics, including solubility and molecular weight (Mw), lately been shown to be crucial for their physiological effects.[3,4] The most important chemical compounds in contemporary medicine are polysaccharides, and β -glucan is the most popular and changeable metabolite with a broad range of biological action. A soluble fiber called β-glucan is generated from the cell walls of plants, bacteria, fungus, yeast, and algae. The structure, solubility in water, Mw, and size of β-glucans derived from mushrooms vary. They display a variety of health-improving advantages.[5,6] Although β-glucans in grains (such as oats and barley) include 1, 3 and 1, 4 links, those in yeast and mushrooms possess 1, 3 β-glucan connections and sometimes 1, 6 β-glucan connections.[7] The β-1,3/1,6 glucan produced from yeast is said to have more biological activity than its 1,3/1,4 bounds. The solubility of β-glucans affects their

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efficacy.[8] Oyster mushrooms are one of the most significant resources of β-glucans, claim Rop *et al*. Plant hemicellulose polysaccharides (soluble fibers) known as β-glucans are understood to be hypocholesterolemic substances.[9,10]

The aim of the study was to obtain β-glucan from oyster mushroom by alkaline KOH extraction, to characterize β-glucan in terms of structure, viscosity, Mw, and rheological properties.

MATERIALS AND METHODS

Oyster mushrooms were of variety *Pleurotus pulmonarius* cultivated in Kütahya and were purchased from a local glocery store in Kütahya. Analytical grade chemicals were obtained from Sigma-Aldrich Co. or from Merck.

Extraction of β-Glucan from Mushroom

With a few adjustments, the polysaccharide was isolated from Yap and NG using the technique.[11] Initially, 400 g of fresh osyter mushroom fruiting bodies were cleaned and cut into little pieces. The samples of entire mushrooms were heated with KOH after being cooked in 80% ethanol reflux. Alkali was used by Wei *et al.* to extract β-glucan.[12] The oyster mushrooms was refluxed with 80% ethanol for 6 hours. Next, the mushroom was extracted with different concentrations of KOH (10%, 20%, 30%, and 40%), at different temperatures (50, 60, 70, 80, and 90°C), and different times (30, 60, 90, 120, and 150 min). It is considered that three factors KOH content, extraction temperature, and time are in the interim. With the help of Whatman Nr1 filter paper, the extract was filtered and then concentrated in a rotary evaporator operating at 60°C at decreased pressure. At the same time, the Sevag reagent (chloroform/butanol, 4:1) removed the proteins in the extract. The concentration was increased by three times by the addition of 95% ethanol. 1500 g centrifuged at 20°C for 10 min. The pH of the supernatant was adapted to 10 applying Na_2CO_3 after 20 min of centrifugation at 22000 g at 4°C. A 1:2 mixture of 80% ethanol and supernatant was maintained for 15 min. The pellets from a 3500 g centrifugation at 4°C are dried in a vacuum oven.

Nuclear Magnetic Resonance (NMR) Analysis

According to the procedure outlined by Zhu *et al.* (2010), the polysaccharide samples (30 mg) were ultrasonically dissolved in D_2O for 30 min before being subjected to ^1H and 125.75 MHz (^{13}C) measurements.[13]

The inability to identify undissolved materials made it difficult to determine the insolubility of particulate β-glucans during NMR analysis. To clarify the glucan conformation, a high resolution ^{13}C-NMR of β-glucan particles was carried out in solid state. Powdered β-glucan particles were inserted onto a 3.2 mm Zirconium rotor for NMR examination. A 600 MHz NMR spectrometer was used to record all NMR spectra. Using a Bruker 3.2 mm DVT probe and operating at frequencies of 600.154 MHz for ^1H and 150.154 MHz for 13C. All studies used a magic angle spinning (MAS) frequency of 10.0 kHz. The precision of the Bruker MAS pneumatic unit's spinning speed control was 2Hz. All tests will employ a 5 s cycle delay. Each cross-polarization (CP) experiment required a total acquisition time of 0.017 s. The spectral width used to acquire all ^{13}C CP-MAS NMR spectra was 315 ppm.[14]

Liquid-chromatography-mass Spectrometry (LC-MS) Analysis

Agilent 1290 rapid resolution liquid chromatography series equipped with 6550 iFunnel Q-TOF LC/MS system was used to conduct the analysis. From the lowest level of concentration to the greatest level of concentration, β-glucan standards were run. As a blank, pure Milli-Q water was utilized.[15]

Preparation of Solutions and Rheological Measurement

Several β-glucan concentrations were made by dissolving dried β-glucan powder in distilled water, which was then stirred at 25°C for 60 min on a hot plate magnetic stirrer. The shear rate ramp test was performed employing a cone-and-plate dynamic rheometer with a 40 mm diameter, a 4° cone angle, and a 0.1425 mm gap (Kinexus Dynamic Rheometer, Malvern, UK). Shear rate ramps with a total ramp length of 2 min and 20 sample points were used to detect shear stress at shear rates ranging from 0.1 s^{-1} to 100 s^{-1}.[16]

RESULTS AND DISCUSSION

With more N- and O-type carbohydrate-peptide connections were broken as KOH concentration rose, which led to the release of more polysaccharide. Nevertheless, the structure of the polysaccharide was harmed when the KOH concentration rose over 30%. Protein denaturation and a decrease in extraction rate were both caused by high temperatures. Another element influencing polysaccharide extraction is extraction time. Polysaccharide degradation was brought on by prolonged extraction time. Hence, 90 min was the ideal extraction time. Table 1 lists the reported findings for various extraction settings. Based on the rate of polysaccharide extraction, alkaline extraction was chosen as the optimum technique. The extraction procedure was carried out at increasing KOH concentrations (0, 10, 20, 30, and 40%) for increasing periods at increasing incubation temperatures (50, 60, 70, 80, and 90°C) and at increasing duration of time (30, 60, 90, 120, and 150 min). In the meanwhile, three variables, we considered KOH concentration, extraction temperature, and extraction time. Three times each element was checked. The extract was filtered using Whatman Nr1 filter paper, and the filtrate was then concentrated in a rotary evaporator operating at 60°C at reduced pressure. Sevag reagent was used to extract the proteins from the extract, and after Sevag reagent was removed, the concentrate was mixed with three times its volume of 95% ethanol before being left to stand at 4°C overnight to precipitate polysaccharides. To extract crude polysaccharides by lyophilization, the precipitate was collected after being centrifuged at 3000 rpm for 10 min. It was then dialyzed with flowing water for 48 h.

Table 1: Extraction yield%

% KOH	Temperature (°C)	Time (min)				
		30	60	90	120	150
10%	50	0.025	0.0375	0.05	0.05	0.125
	60	0.125	0.05	0.0625	0.055	0.175
	70	0.137	0.06	0.075	0.0875	0.225
	80	0.175	0.077	0.0875	0.0925	0.275
20%	50	0.055	0.05	0.075	0.1	0.125
	60	0.075	0.10	0.11	0.125	0.275
	70	0.075	0.11	0.125	0.137	0.3.
	80	0.125	0.15	0.175	0.187	0.325
30%	50	1.25	1.75	**2.55**	2.25	2.
	60	1.50	2.00	**3.90**	2.5.	2.5
	70	1.75	2.00	**4.20**	2.25	2.53
	80	2.25	2.37	**5.50**	3.12	2.52
40%	50	1.25	1.50	2.37	2.12	1.75
	60	1.40	1.75	2.45	2.25	2.00
	70	1.50	2.25	2.55	2.25	2.00
	80	2.00	2.25	2.50	2.00	1.75

Bold values denote maximum extraction yield (%)

In conclusion, 30% (w/v) KOH concentration, 80°C, and a 90-min extraction time were the ideal extraction parameters. Hence, 90 min was the ideal extraction period. The isolated solution's protein was eliminated using the Sevag reagent.[17] Only by isolating purified β-glucan from the plant matrix using various extraction methods is it possible to do so. Several extraction stage pHs, such as acidic, neutral, and alkaline, may be used in wet extraction methods. Alkaline water processes provide the best yield of extracted β-glucan despite the fact that the process has distinct properties.[18-20] At neutral pH and notably alkaline pH, a simultaneous rise in protein solubility was seen.[21,22]

The alkaline extraction method yielded the highest yield and purity, ensuring maximum removal of impurities. In addition, this method resulted in the highest amount of soluble β-Glucan. In a conducted study, it was determined that a NaOH concentration above 0.5 M, extraction temperature of 135°C, and extraction time of 45 min were the ideal extraction parameters.[23] These ideal extraction parameters were further confirmed by Fourier transforms-ınfrared spectroscopy (FTIR) analysis [Figure 1].

FT-IR Analysis

The functional groups such as OH-, C-H, and C-O of the crude β-glucan were identified using FT-IR. The absence of water-soluble glucan absorption bands in the infrared spectrum, which are characteristic of β-(1--3)/(1--6)-glucans. In Figure 1, the FTIR spectrum is shown.

The linkages in the area of 3301.906 cm^{-1}, 2921.754 cm^{-1}, 1639.486 cm^{-1}, and 1368.359 cm^{-1} in β-glucan were typical polysaccharide absorptions [Table 2]. Stretching of the OH- bond was ascribed to the band at 3301.901 cm^{-1}. The bending modes of C-H and OH gave rise to the following bands at

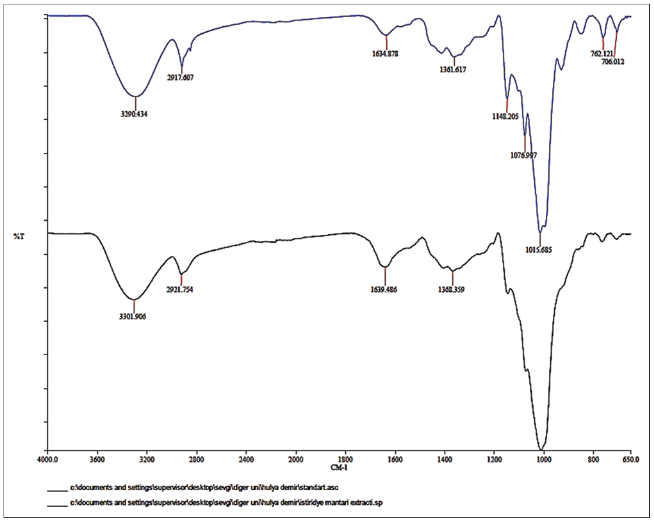


Figure 1: Fourier transforms-ınfrared spectroscopy analysis of sample and standard

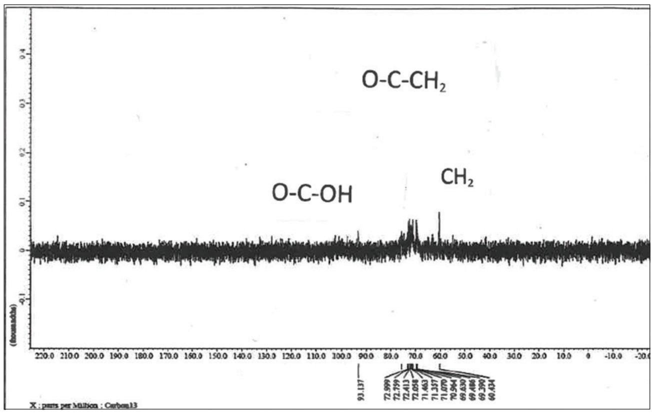


Figure 2: ^{13}C nuclear magnetic resonance spectrum β-glucan sample (a)

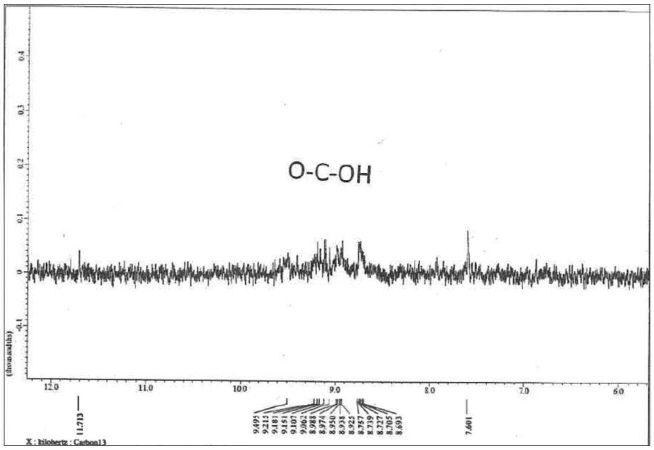


Figure 3: ^{13}C nuclear magnetic resonance spectrum β-glucan sample(b)

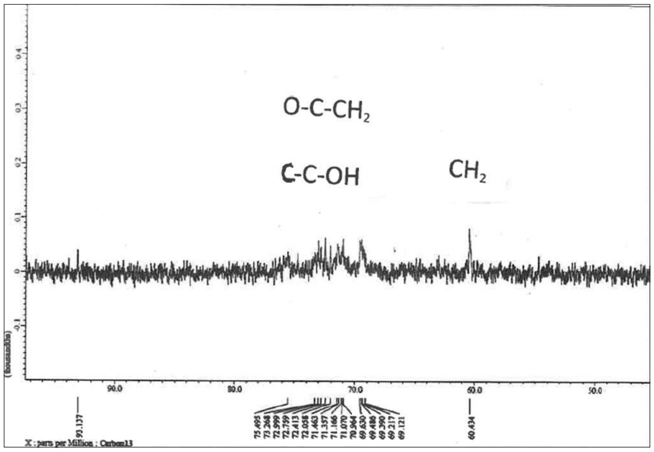


Figure 4: ^{13}C nuclear magnetic resonance spectrum β-glucan sample (c)

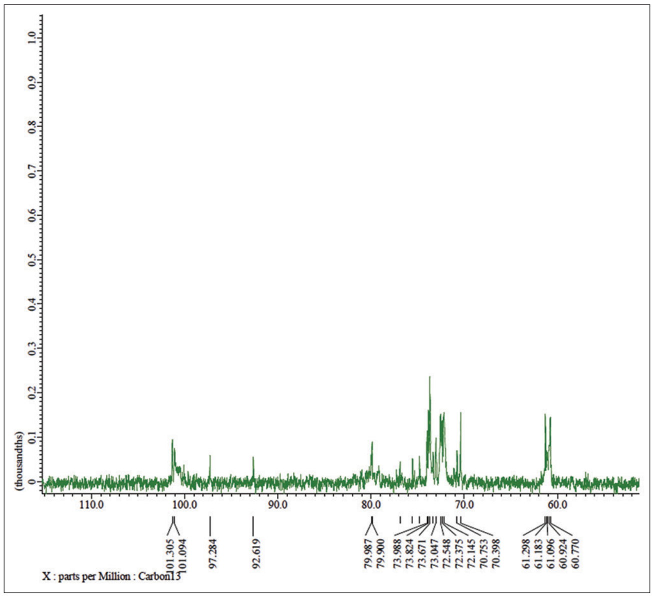


Figure 5: ^{13}C nuclear magnetic resonance spectrum β-glucan standard (a)

2921.754 and 1639.486 cm^{-1}. Many bands below 1015.685 cm^{-1} belong to ring and skeleton modes, whereas intensely overlapped bands in the range of 1015.685 cm^{-1} relate to CO, CC stretching, and COH bending modes. The presence of a band at 890 cm^{-1} is a distinct characteristics of polysaccharides with a β-configuration, specifically indicating the (C1-H) deformation mode. In addition, there are absorption bands at 2920, 1370, 1250, and 1200 cm^{-1} that differentiate 1---3 linkages. The absorption at 1160 cm^{-1} corresponds to (C-C) and (C-O-C) stretching vibration, while two partially overlapping bands at 1078 cm^{-1} and 1048 cm^{-1} are associated with ring stretching and (C-OH) side group stretching, respectively.[24]

Free hydroxyl groups absorb in the region of 3301.906 cm^{-1}. The hydroxyl groups involved in hydrogen bond formation caused the maximum

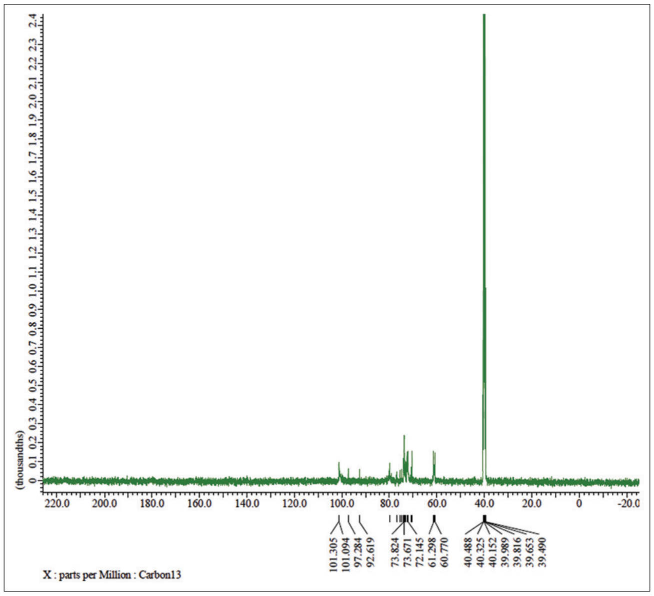


Figure 6: ^{13}C nuclear magnetic resonance spectrum β-glucan standard (b)

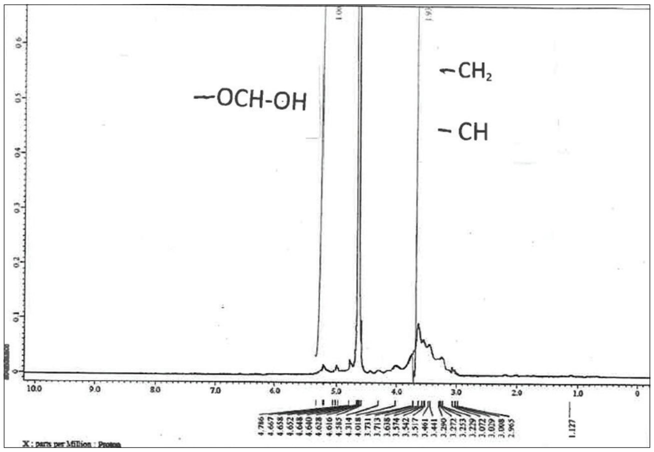


Figure 7: ^{1}H- nuclear magnetic resonance spectrum β-glucan sample (a)

Table 2: The content of β-glucan

Bond area	Functional group
3301.906 cm^{-1}	OH
2921.754 cm^{-1}	CH
1639.486 cm^{-1}	OH
1368.359 cm^{-1}	CH_2, CH, OH
1015.685 cm^{-1}	CO, CC, COH (β-anomers of glucans)

Table 3: Chemical shifts of signals in ^{13}C NMR spectra of glucan

Carbohydrate residues	Chemical shifts, ppm					
	C1	C2	C3	C4	C5	C6
3,6-β-Glc-(1-3)	93.137	7.413	72.159	69.486	73	69.63

NMR: Nuclear magnetic resonance

position of the band to shift to lower frequencies, increasing its intensity and broadening the band, while at the same time, also caused symmetry distortion.

NMR Spectroscopy

To reveal the conformational properties of crystalline as well as non-crystalline samples, high-resolution solid-state ^{13}C NMR spectroscopy has recently proven to be very useful for various types of molecular systems.[25]

Analyses were taken under room temperature using a standard 5 mm NMR tube at the JEOL ECZ 500 R NMR device. The sample was dissolved in D_2O. The number of screening was adjusted as 32 and the clarification time as 5 s.

According to the ^{1}H NMR spectrum of the sample, it was observed that there were impurities in the sample. However, a trace of humidity was also present in the sample. Besides all of them, the solubility of the sample in D_2O was quite low. Due to the fact that the analyses were taken in the D_2O solvent, the protons of the OH groups in the structure exchanged protons with the Deuterium atoms in D_2O, thus OH peaks could not be observed in the ^{1}H NMR spectrum. In addition, it was thought that the protons of the CH_2 group structure reached at 3.638 ppm. The protons of the CH groups within the group came quite close between 3.1 ppm and 4.0 ppm. CH groups were also close to each other due to the fact that they were remarkably similar in terms of their chemical environment. CH peak in O-CH-OH group was thought to have come at 5.32 ppm. Other incoming peaks came from the impurities in the environment.

According to the ^{13}C NMR spectrum of the sample, the peak of the C atom in O-C-OH group was observed at 93.137 ppm (11.713 kHz), and the peak of the C atom in O-C-CH_2 group was observed at 75.495 ppm (9.495 kHz) [Table 3]. The peaks of the C atoms in three different C-C-OH groups in the structure were observed at 72.999 ppm (9.181 kHz), 72.413 ppm (9.107kHz), and 72.058 ppm (9.062 kHz), respectively. The peak of the C atom of CH_2 group was detected at 60.434 ppm (7.601 kHz) [Figures 2-4]. All of the monosaccharide residues in oyster mushrooms have anomeric configurations, as shown by resonances of 93.137 ppm that are assigned to anomeric carbon atoms [Figures 5 and 6]. There are no carboxyl, acetyl, or amido groups in β-glucan since no chemical change was seen over 170 ppm.

The water-soluble glucan's ^{1}H NMR spectrum is shown in Figures 7 and 8. Due to the existence of the macromolecule's -anomeric form, a strong peak (4.5 ppm) in the spectrum is seen. These results agree with those from FTIR spectroscopy.

Proton magnetic resonance chemical shifts between 3.00 and 4.50 ppm show that this polymer is a carbohydrate-based molecule [Figures 9 and 10]. The existence of ^{1}H, 1-3-β-linked glucopyranose and 4.36 ppm ^{1}H, 1-6- β-linked glucopyranose is indicated by proton signals in the spectra of anomeric carbon atoms at 4.47 ppm and 4.36 ppm -^{1}H, respectively [Figure 8]. Its structure is the same as that of other D-(1--3)/-(1--6)-β-glucans. Its primary chain is made up of linked (1–3)-glycoside linkages made from the residues of D-glucopyranose.

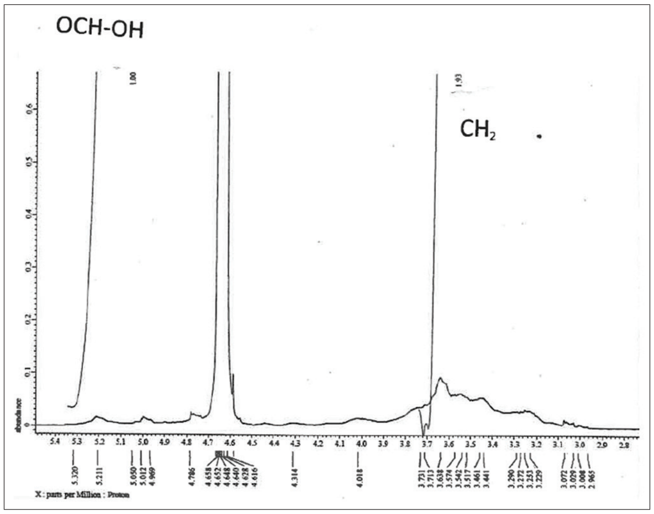


Figure 8: ^{1}H- nuclear magnetic resonance spectrum β-glucan sample (b)

The data obtained were similar to those described in the literature.[25] Based on the results obtained, it was concluded that the water-soluble glucan macromolecule has a branched structure, the basis of which consists of D-glucopyranose residues linked by β-(1→3)-glucoside bonds. Lateral branches, built from β-glucopyranose monomer units, were attached to the O-6 positions of monosaccharide residues.

In the current study, the proton magnetic resonance chemical shifts in the range of 3.00–5.35 ppm suggest that the polymer being investigated possesses a carbohydrate structure. The presence of proton signals in the spectrum of anomeric carbon atoms at 4413 ppm indicates the presence of ^{1}H, β-1→3- linked glucopyranose, and at 4018 ppm ^{1}H β-1→6-linked glucopyranose. Results are consistent with the literature.[26-28]

LC-MS Analysis

In our study, we found the Mw of β-glucan to be 349.260 g/mol. In addition, the presence of (1—6) branch chains is an important result. It increases the biological activity of β-glucan. β-glucan with a Mw

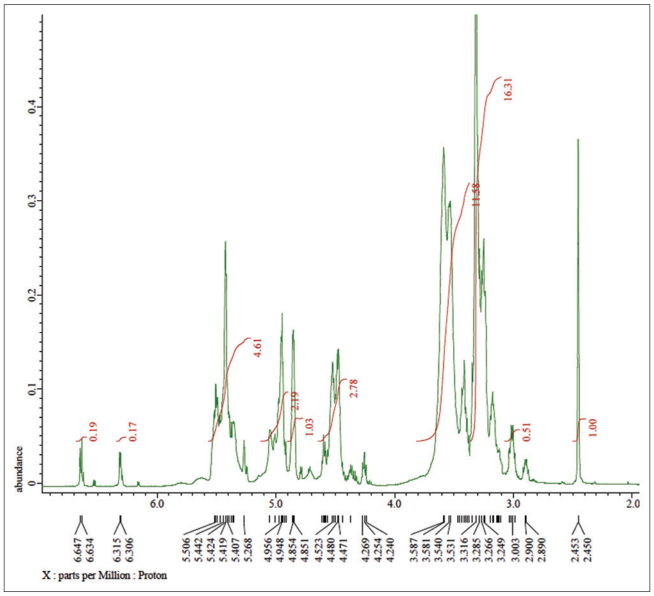


Figure 9: ^{1}H nuclear magnetic resonance spectrum β-glucan standard (a)

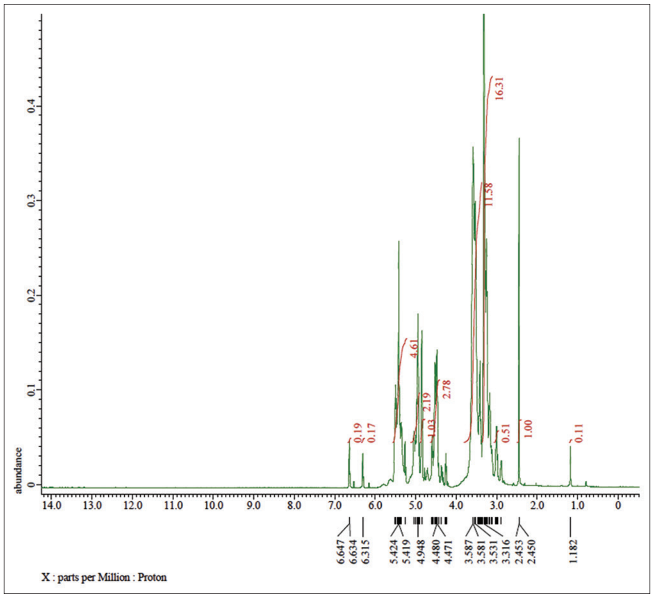
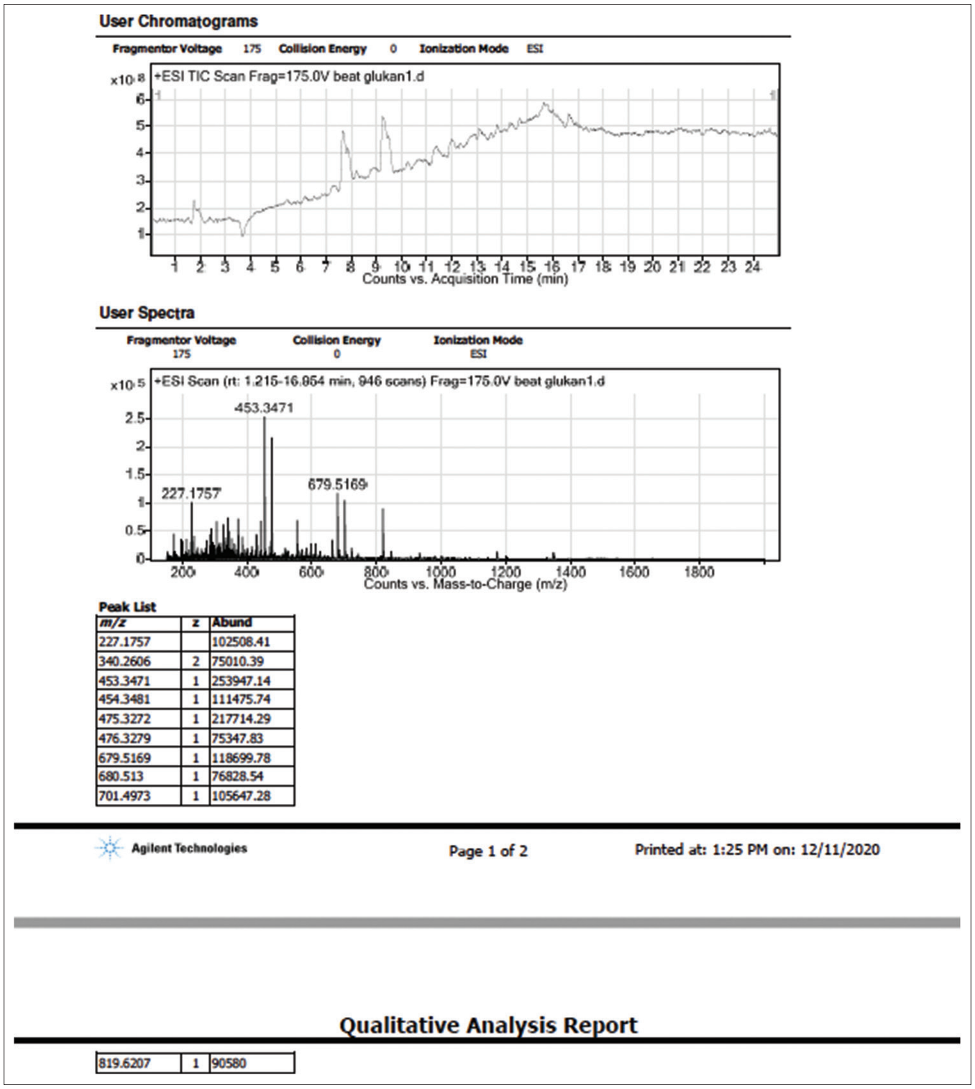


Figure 10: ^{1}H- nuclear magnetic resonance spectrum β-glucan standard (b)



User Chromatograms

Fragmentor Voltage 175 Collision Energy 0 Ionization Mode ESI

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
175	0	ESI

Peak List

m/z	z	Abund
227.1757		102508.41
340.2606	2	75010.39
453.3471	1	253947.14
454.3481	1	111475.74
475.3272	1	217714.29
476.3279	1	75347.83
679.5169	1	118699.78
680.513	1	76828.54
701.4973	1	105647.28
819.6207	1	90580

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Qualitative Analysis Report

Figure 11: Liquid-chromatography-mass spectrometry chromotograph analysis sample β-glucan (b)

of 349.260 g/mol has more mobility and is more able to form junctions with near chains and then easily rearranging itself. With this property, beta-glucan with 300 g/mol extensively used to prepare pseudoplastic solution in food industries.[29] In general, beta-glucan with high Mw is more effective in biologically in comparison to beta-glucan with low Mw.[29] Finding the Mw of β-glucan as 349.260 g/mol in our study is a good result [Figure 11]. Another factor is the degree of branching of β-glucan that affects the biological activities of β-glucan.

Another important effect of β-glucan is its antihyperglycemic effect. The Mw of β-glucan adjusts the glycemia status. In one study, a beverage containing oat β-glucan (5 g) with a Mw of 100 g/mol provided lower postprandial glucose and insulin levels than a beverage containing barley β-glucan (5 g) with a Mw of 400 g/mol. In research with beta-glucan obtained from Saccharomyces cerevisiae, it has been shown that this product can be used as a nutraceutical as well as to lower blood sugar and reduce pain in diabetic patients.[30] Kim *et al.* (2009), showed that regularly consumed barley grains containing beta-glucan reduced insulin response.[31]

Rheological Properties

The shear stress (τ) versus shear rate (γ) data for β-glucan solutions were fitted well to Herschel-Bulkley model at 25°C (Eq. 3.1).[32]

$$\tau = \tau 0 + k\ (\gamma) n \qquad (3.1)$$

In the equation, shear rate is denoted by τ (s^{-1}), yield stress is denoted by τ^0 (Pa), consistency coefficient is denoted by k (Pa.s^n), and flow behavior index is denoted by n.

For all the samples in Table 4, τ^0 (Pa) represents yield stress, and k (consistency coefficient) and n (flow behavior index) values were calculated with Statistica program by applying Herschel-Bulkley model. The range of the flow behavior index (n) is 0.99751 to 1.0251. The greatest n value for β-glucan concentration at 0.6% was 1.0251, indicating that it behaves like a Newtonian fluid ($n = 1$).[32]

High concentrations of water-binding macromolecules including fibers, emulsifiers, and hydrocolloids are thought to improve how well baked foods combine and extend their shelf life. The enhanced water holding capacity of β-glucan solutions may be the cause of the greatest yield stress and consistency index values.[33]

Since yield stress could not be determined at each concentration, the Power-Law model was used. Power-law flow modeling of fluids; the most common approximation method used to describe the viscous flow behavior of non-Newtonian fluids (such as polymer melts) uses a Power-Law relationship.

$\tau = K\dot{\gamma}^n$ (Ostwald de Waele model)

Where K is the consistency coefficient (Pa.s^n)

n is the flow behavior index (dimensionless), γ (s^{-1})= shear rate

If n=1, then K=η=viscosity

For pseudoplastic fluids, n < 1

For dilatant fluids, n > 1

Yield stress was enhanced in parallel with an increase in β-glucan concentration, and the highest value of yield stress was observed in 0.8% concentration of β-glucan. Similar findings have been reported by Bhatty, Dawkins and Nnanna, Burkus and Temelli, Colleoni *et al*., and Ghotra *et al*., for β-glucan of mushroom, barley bran, oat, barley, and high purity barley b-glucan, respectively.[19,34-37] According to Szczesniak and Farkas, the presence of yield stress in certain hydrocolloids can be attributed to intramolecular interactions. For example, the interaction of acetate groups in xanthan gum leads to the development of yield stress. In contrast, β-glucan lacks charged groups that contribute to such interactions. Therefore, the strengthening of hydrogen bonds at higher concentrations could potentially be the cause of yield stress. Yield stress is a valuable and practical property of hydrocolloids, particularly when they are employed as viscous agents in food formulations. It helps to safeguard the structure of food against stress and temperature variations.[38]

Table 4: Herschel-Bulkley parameters of β-glucan at 25°C

Concentration (%) β-glucan	τ0 (Pa)	k (Pa.s^n)	*n*
0.8	$6.4122.10^{-3}$	0.0023813	0.99751
0.6	$1.7218.10^{-3}$	0.0020619	1.0251
0.4	cannot be calculated	0.002209	0.99957
0.2	$0.51346.10^{-3}$	0.0020312	1.0094

For all the samples in Table 5, R^2 (coefficient of determination), K (consistency coefficient), and n (flow behavior index) values were calculated with Statistica program by applying Power-Law model. The present study was carried out to investigate flow behavior of β-glucan, as influences of concentrations (0.8%, 0.6%, 0.4%, and 0.2%) temperature 25°C. Shear stress data were fitted versus shear rates using the model. Since our sample is a polymer solution, its viscosity varies depending on the shear rate. Since it does not have a fixed viscosity, it must fit a model. Shear shows thinning behavior. While it is a solid sample at rest, it becomes thinner and more liquid as the mixing or pumping process accelerates. Shear-thinning fluids, also known as pseudoplastic fluids, exhibit a decrease in viscosity with increasing shear rate. Polimer solutions and other solutions containing high Mw compounds are typical examples of pseudoplastic fluids [Figure 12]. These fluids will develop shear stress when the shear rate is modest. The molecules reorganize as a result of the shear stress to lessen the total stress. The viscosity of polymer solutions increases as the increase of the Mw of the polymer increases since longer chains occupy more space and increase the

Table 5: Effect of 0.8%, 06%, 0.4%, and 0.2% concentrations on consistency (K), flow index (n), and correlation coefficient of power law model (R^2)

Concentration (%) β-glucan	K (Pas^n)	n	R^2	SD
0.8	7.5825×10^{-3}	0.68977	0.93467	0.015917
0.6	2.6044×10^{-3}	0.96568	0.99827	0.0025671
0.4	2.3354×10^{-3}	0.98239	0.9995	0.0013106
0.2	2.252×10^{-3}	0.98184	0.9993	0.0014983

resistance to flow. As a biopolymer, the Mw of β-glucan affects the viscosity.[39]

Figures 13 and 14 shows how the viscous (loss) (G”) and elastic (storage) moduli (G’) work of β-glucan remedies. The findings showed that values increased in all samples with increasing frequency. These powder suspensions exhibit viscoelastic behavior, which describes their dual viscoelastic properties.

These β-glucan solutions have viscoelastic behavior, meaning they have both viscosity and elastic elasticity. All samples’ elastic modulus values were discovered to be higher than their viscous modulus values, demonstrating the solid behavior of the β-glucan solutions. Moreover, the elastic and viscous moduli of β-glucan solutions were high. Greater shear rates are produced by β-glucan solutions over longer periods of time, which leads to the formation of more hydrophilic moieties and higher modulus values. By maintaining a consistent pressure throughout the procedure, β-glucan solutions create finer particles.[40]

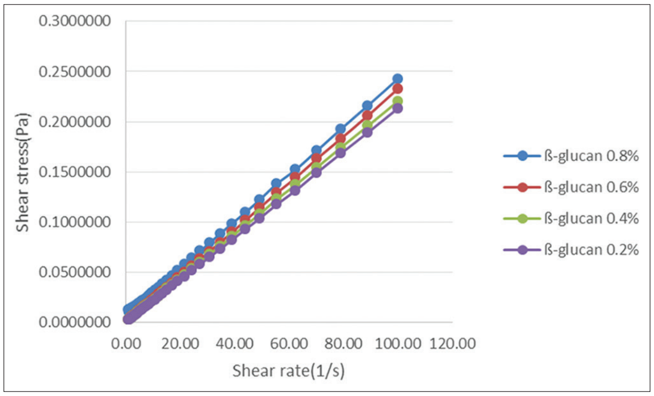

Figure 12: Flow curves obtained for 0.2%, 0.4%, 0.6%, and 0.8% β-glucan solution at 25°C

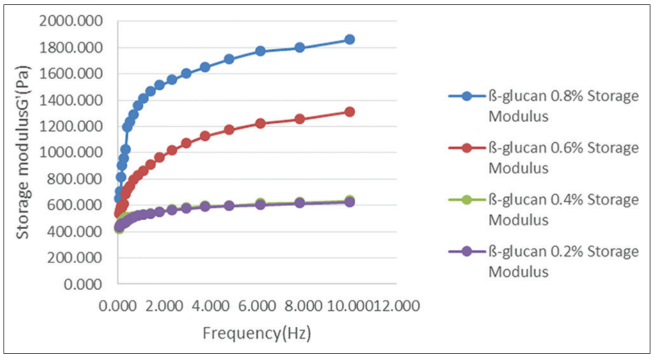

Figure 13: Storage modules (G´) obtained for β-glucan solutions at different concentrations. G´at 0.2%, G´at 0.4%, G´at 0.6%, and G´at 0.8% solution

Figure 13 demonstrates that every sample exhibits the same pattern. a distinction between G’ and G” when the water content rises, values fall. When dispersions are diluted with water, the sample tends to exhibit characteristics more akin to a viscous liquid rather than an elastic solid, which is in line with expectations. In the study, an oil/water interfacial film was formed using gum arabic and modified starch and frequency shear test was performed on the film. As a result of the studies conducted at 20% and 10% concentrations of gum arabic, higher elastic properties were obtained at 20% concentration, and viscous properties were found to be dominant in the 10% sample. Thus, it was stated that especially the elastic modulus properties increased as the concentration increased.[40]

Three duplicates of the shear stress measurements for each shear rate were made, and their average results were computed. To assess whether model performs better in predicting the rheological behavior of β-glucan, R^2 and root mean square error (RMSE) were determined. As a rule, the model with the greatest R^2 and lowest RMSE without any estimated parameter values with negative values was chosen as the best model. Power-Law was the best model for fitting shear stress data versus shear rate, despite models with yield stress having high R^2 (>0.99) and low RMSE.

The fitting of shear stress versus shear rate data by power law model indicated that β-glucan of mushroom shows non-Newtonian behavior in different concentrations. Flow behavior index (n) <1 for all concentrations suggests pseudoplastic behavior (shear-thinning behavior). Colleoni-

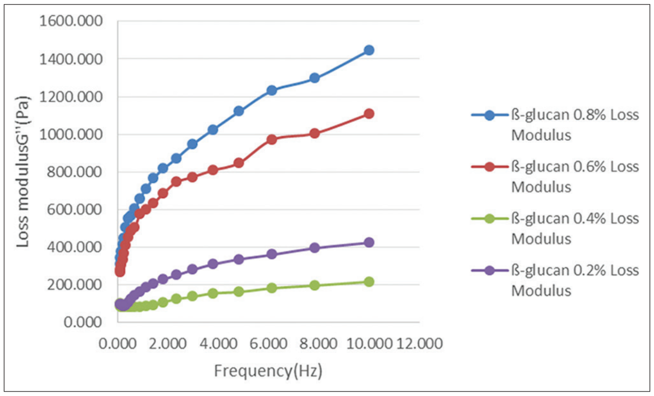

Figure 14: Loss modules(G’’) obtained for β-glucan solutions at different concentrations. G’’ at 0.2%, G’’ at 0.4%, G’’ at 0.6%, and G’’ at 0.8% solution

Sirghie *et al.* (2003), reported that by increasing oat β-glucan concentration from 0.25 to 1%, the flow behavior index was decreased from 0.855 to 0.504.[41]

Hydrocolloid solutions with a high flow behavior index ($n > 0.7$) cause an unfavorable slimy feeling in the mouth and are suitable as fat replacer in foods. Therefore, for providing high viscosity and good mouthfell, hydrocolloids with a low flow behavior index should be used.

In dynamic tests, the main viscoelastic properties are the storage (G') and loss (G'') moduli which allow the calculation of $\tan\delta = G''/G'$. In this work, these properties were mainly determined by frequency sweep tests. Temperature scanning tests were not performed because this system does not show thermal transitions.

According to Steffe, materials usually exhibit more solid-like characteristics at higher frequencies.[40] This means that a G'' and G' crossover would be expected around high frequencies, as observed by Torres *et al.* studying gels based in Chitosan crosslinked with glutaraldehyde.[41]

The model is a useful option for forecasting the rheological behavior of hydrocolloids because hydrocolloid components behave as scattered particles in aqueous media. The Power Law model is the most effective one for fitting shear stress data versus shear rate according to Koocheki *et al.* (2013), solution of *Lepidium perfoliatum* gum.[42]

All β-glucan solutions regardless of concentration have a shear thinning tendency, as shown in Figure 3.8 and 3.9 for the Dynamic and Power law models. The flow index fell as the β-glucan concentration rose, demonstrating that the solution's pseudoplasticity was boosted by raising the β-glucan concentration. The ideal design of food processing systems may benefit from knowledge of yield stress.[43]

According to Szczesniak and Farkas, intramolecular interactions are the cause of yield stress in several hydrocolloids. Hence, the reason of yield stress may be the strengthening of hydrogen bonds at greater concentrations. In particular, when hydrocolloids are utilized as viscosity agents in food forms, preserving the food structure from stress and temperature change, yield stress is a valuable and practical attribute of hydrocolloids.[38]

The findings demonstrated that the consistency coefficient grew non-linearly as the content of β-glucan rose. This is most likely caused by the molecules' increased ability to retain water. Increasing hydrocolloid concentration boosts intramolecular interaction and entanglement, which raises the consistency coefficient and apparent viscosity. These effects are accompanied by a rise in consistency coefficient. Similar findings on the impact of concentration on the improved consistency coefficient of salep and *Alyssum homolocarpum* seed gum were published by Koocheki (2013) correspondingly, *L. perfoliatum* seed gum.[42]

While under stress, a material's rheological qualities lead it to flow and deform. No matter how quickly the shear rate varies, the viscosity of Newtonian fluids stays constant. Depending on the kind of fluid, the viscosity of the fluid may either grow or decrease when the shear rate is raised. The viscosity decreases as the shear rate increases for fluids that are shear thinning, but the converse is true for fluids that are shear thickening. If the force is not great enough to break the plastic, it would not flow. Time-dependent fluids have viscosities that change over time. Foods that are both elastic and viscous are known as viscoelastic foods. Tests for dynamics, creep, and stress relaxation may all be used to find materials that have viscoelastic qualities. The characteristic resistance to motion of a fluid is referred to as its "viscosity."

Non-Newtonian fluids are fluids that do not adhere to Newton's viscosity law. Fluids that are shear thinning or thickening follow the power law paradigm (Ostwald-de Waele equation) fluids that shear thin (or are pseudo-plastic). Layer-to-layer friction in these fluids diminishes as shear rate rises. Viscosity is decreased through shearing, which forces entangled, long-chain molecules to straighten up, and align with the flow. Paint is a common illustration of a fluid that shears thins.

When paint is put to a surface without using a brush, its viscosity rises, and it is unable to flow due to gravity. The viscosity of paint diminishes when it is applied to a surface using a brush that shears the paint. Apple sauce, banana puree, and concentrated fruit juices are a few examples of fruit and vegetable products that are ideal examples of pseudoplastic fluids in food systems. Krokida *et al.* (2001) looked into the rheological characteristics of fruit and vegetable products. Although the flow behavior index n somewhat dropped with concentration, the consistency coefficient k grew rapidly. For pulpy goods, the flow behavior index was near to 0.5, while for clear juices, it was close to 1.0.[44] Foods' rheological properties may vary as they get more concentrated. Shear thinning was seen in the rheological behavior of concentrated grape juice with a Brix value of 82.1. Yet it was discovered that diluted samples with Brix values ranging from 52.1 to 72.9 were Newtonian. In a staple food item in Turkish breakfast, sesame paste-concentrated grape juice blends were examined for their rheological behavior at two different sesame paste concentrations: 35% to 65°C and 20% to 32°C. With a flow behavior index ranging from 0.70 to 0.85, all of the mixes demonstrated shear thinning behavior.[45]

The storage modulus (G') and loss modulus (G") of the samples, respectively, were used to describe their elastic and viscous characteristics. Plot the samples' loss and storage moduli versus frequency and display them. Differentiating between a solution and a gel-like structure is possible thanks to the slopes of the storage modulus (G') and the loss modulus (G"). The similar response was shown in the situations of β-glucans 0.2% and 0.4%. The samples with the greatest and lowest elasticity (G') were 0.8% and 0.2% of β-glucan, respectively. This tendency was only apparent in the samples of β-glucan 0.2%, 0.4%, 0.6%, and 0.8% in the current investigation.

In certain circumstances, a decline in the storage modulus (G') values at higher frequencies may indicate that the network structure has broken apart into chains. All samples' storage moduli (G') rose with frequency before reaching a plateau. The plateau values of G' may be utilized to determine the networks' tensile strength and elasticity.[46]

According to one definition, a gel is a substance in which G' has a positive slope and G' > G" at 6 Hz.[46] The mechanical spectra of β-glucan solutions began to first adopt elastic networks (G' > G") for β-glucan 0.2%, 0.4%, 0.6%, and 0.8% samples. For samples of 0.2%, β-glucan 0.4%, β-glucan 0.6%, and β-glucan 0.8%, the loss modulus response (G") rose with increasing frequency. Although G' was always greater than G" in the solutions of the 0.2%, β-glucan 0.4%, β-glucan 0.6%, and β-glucan 0.8% samples, these samples exhibited elastic characteristics.

Skendi *et al*., extracted isolated β-glucan from two oat varieties' ground seeds. All of the β-glucan samples tested were capable of generating gels, according to dynamic rheometry. In contrast to their counterparts with high Mw, the low Mw oat β-glucan exhibited high gelation rates and rapid gelation periods. It was previously noted that the low-Mw β-D-glucan gels up more quickly than the high-Mw β-D-glucan. Low-Mw β-glucan's high mobility and high structural regularity make junction zones and three-dimensional networks simple to form, which in turn speeds up gelation.[45] The solution becomes more viscous due to β-glucan. A distinctive quality of the polysaccharide solution is also thought to be its inherent viscosity. According to some theories, the concentration, capacity to form aggregates, and molar mass of the polysaccharides all affect viscosity. Many investigations have shown the strong relationship between the Mw of β-glucan and solution viscosity. Kim and White (2010) also noted that the viscosity increased when the Mw of oat β-glucan increased.[47] Moreover, it has been shown that gamma irradiating β-glucan causes the radiolysis of glycosidic bonds, which lowers its Mw and subsequently lowers its viscosity.[46]

Viscosity; among the samples, variations in slurry viscosities were noted. Variations in polysaccharide's Mw and solubility cause variations in viscosity. Figure 15 show the apparent viscosities of samples containing β-glucans at various shear rates.

Shear thinning characteristics are also primarily influenced by variations in Mw and the fine structure of β-glucan. The viscosity of each sample usually decreased as the shear rate increased. The shear applied causes the molecular entanglements of the β-glucan to be broken, which results in the shear thinning flow characteristic. According to reports, Mw influences when shear thinning behavior starts.

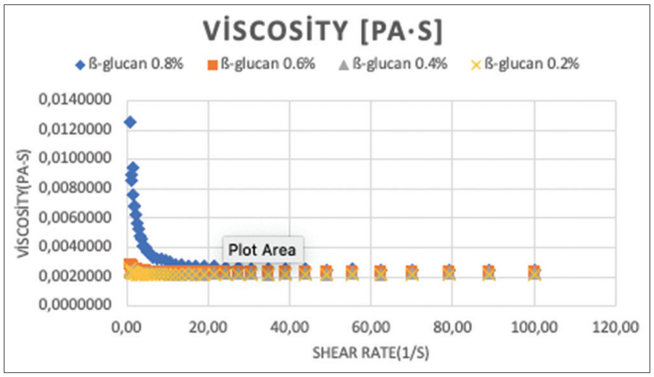

Figure 15: Shear rate dependence of viscosity for 0.8, 0.6, 0.4, and 0.2% β-glucan solution

According to Lazaridou *et al.* (2007), the β-glucans solutions exhibit high apparent viscosities at low shear rates and a shear thinning flow at high shear rates. Smaller polymers have been suggested to increase the degree of aggregation because of their higher concentrations.[47] The rate of diffusion may increase viscosity. As β-glucan competes with starch for water, Faraj *et al.* (2006) found that the purity of the β-glucan was a key factor in the viscosity of β-glucan starch blends.[48] Nevertheless, Papageorgiou *et al.* (2005) found a modest correlation between total β-glucan concentration and slurry viscosity.[49]

CONCLUSION

In this study, (1–3) (1–6) β-glucan was obtained from oyster mushroom by KOH alkaline extraction, characterized, and applied to β-glucan model food. The ideal extraction process parameters were found as 80°C, 90 min, and 30% KOH. ^{1}H and ^{13}C NMR spectroscopy was used for chemical characterization, and extracted β-glucan was found to be a β-(1-3) glucose polymer with β-(1-6) side chains. These results agree with those from FTIR spectroscopy.

Food having a diverse nature we know that various kinds of solid, semi-solid, and liquid products are available, purely solid we not get that much in case of food only for very low amount of strain this kind of elastic behavior is visible, but mostly fluid and semi-solid nature is widely available. Linear relationship between shear stress and the rate of shear strain governed by the viscosity of the liquid. These relationships are expressed mathematically below.

$$\tau = K\dot{\gamma}^n \text{ (Ostwald de Waele model)}$$

According to shear-thinning behavior, rheological behavior of foods may change depending on concentration. The flow behavior curve has a convex profile in which the tangential slope is decreasing with increasing shear rate. Shearing causes entangled, long-chain molecules to straighten out and become aligned with the flow, reduces friction between two layers and reducing viscosity. Newtonian fluids have the same viscosity at low shear rates as at high shear rates. In constant, non-Newtonian fluids do not have a constant viscosity with respect to shear rate. Rheological measurements showed that β-glucan has low apparent viscosity in aqueous solutions.

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