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Rheological characteristics of *Ganoderma applanatum* exoploysaccharides

Abulaziz Yahya Alghmadi

Biology Department, Faculty of Science, Al Baha University, Al Baha, P. O. Box1034, Saudi Arabia.

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Glucose yeast extract peptone media (GYP) proved to be the most suitable medium for organism growth and exopolysaccharides (EPS) production which recorded 0.320 + 0.01 and 0.1+0.01 (mg/ml) dry weight undershaken and static conditions respectively. Rheological study of *Ganoderma applanatum* exopolysaccharides indicated that viscosity of each EPS concentrations increased with increased shear rate (S.R) values which confirm the dillatent behavior of the different concentrations of EPS. pH value has a valuable effect on the rheological pattern of EPS, with best effect at pH5.5. Furthermore, it was found from the results that sucrose addition as carbon source enhanced the (consistency index cP) viscosity of EPS solution while arabinose decreased EPS viscosity. Therefore, EPS of *G. applanatum* isolated from AlBaha area, Saudi Arabia might have promising applications in field of food and textile industry.

Key words: Growing media, exopolysaccharides, rheology, pH and *Ganoderma applanatum.*

INTRODUCTION

Carbohydrate macromolecules (Glycoproteins, proteoglycans/ glycosaminoglycans, lipoglycans, oligosaccharides and polysaccharides) are the most abundant natural products (Lamari et al., 2003).

Microbial polysaccharides are important since they have many applications in industry and medicine fields. It has been shown that basidiomycete mushrooms contain active antitumor and immunostimulative polysaccharides. Xu et al. (2012) noticed that polysaccharides- protein complex isolated from *Pleurotus pulmonarius* had anticancer activity against liver cancer cells. Also, Cao et al. (2015) reported that polysaccharides extracted from *Pleurotus ostreatus* mycelia markedly reduced both volume and weight of gastric cancer .However, recently there is an evidence from a small number of randomized controlled trials does not support the use of *Glucidum* for treatment of cardiovascular risk factors in people with type 2 diabetes mellitus (Klupp et al., 2015). Current state-to-art researches in this area indicated that published market studies predict that the future of biomass-based polymers is bright, with polysaccharides and polysaccharide-derived polymers being in the forefront. New insights in the structure and functions of polysaccharides in nature open new ways for application developments though polysaccharide engineering. So, it is expecting to see the surge of a deeper understanding of the complexity of biomass, of its bioformation. There is a belief that a new era for polysaccharide science is starting(https://hal.inria.fr/docs/00/57/24/26/PDF/Persin_etal_CarbohydratePolymers2011.pdf).

E-mail: dr-azizghamdi@hotmail.com.

Furthermore, may be over 700 species from higher heterobasidiomycetes and homobasidiomycetes fungi have been discovered to contain active polysaccharides that can be derived from fruit-bodies, culture mycelium and culture broths (Reshetnikov et al. 2001). Zhang and Peng (2003) demonstrated the presence of polysaccharide-protein complex in *Ganoderma tsugae* mycelium. The current research designed to study the production and rheology exopolysaccharides from *G.applanatum* for future applications in different aspects. So, the question is what is the behavior of *G. applanatum* EPS rheological behavior?

Polysaccharides are used as thickeners in the printing and textile industry. The rheological properties of the polysaccharide are important in restricting the flow of the dye. Kim et al. (2003b) studied the influence of pH on reheology during exo-polysaccharides production by *Cordyceps militaris* C738. Yun et al. (2002a) studied the effect of carbon source and aeration rate on carbohydrate rheology during red pigment production by *Ganoderma lucidum* in a batch bioreactor. Fazenda et al. (2010) mentioned that controlling the dissolved oxygen (DO) in the fed-batch culture of the medicinal mushroom *G. lucidum* led to a two-fold increase of the maximum biomass productivity compared to uncontrolled DO conditions. Bae et al. (2000) reported that broth rheology changed during mycelial growth and exo-polysaccharide production through *Cordyceps militaris* fermentation. Morris (1990) clarified that the use of polysaccharides changes by studying the rheological properties of water within foods to modify the texture of foods. Cheen et al. (2002) showed that the viscosity of the expolysaccharides produced from *Cordyceps militaris* gave non-significant high values (100-170cP) and its viscosity is lower than the polysaccharide from other mushroom.

The aim of the current work was to study the production of exopolysaccharide (EPS) from *G. applanatum* culture and study its rheological behavior followed by Brockfield rheometer. Rheological results of the isolated polysaccharides will be manipulated for different applications in food, medicine or textile industry in the near future.

MATERIALS AND METHODS

Studied fungal strains

The studied fungal strain was *Ganoderma applanatum* which was collected from Salix stem trees located in different parts in Alhashas and Khaira villages , Albaha, Saudi Arabia (Figure 1).

Isolation and preservation of fungal strains

Isolation of Ganoderma applanatum:

Fresh collected *Ganoderma applanatum* fruiting body was used to isolate its mycelia from the hymenium layer that was picking up with sterilized forceps. The fresh basidospore part of hymenia was inoculated with sterilized malt agar media (Malt 20 g and yeast extract 1 g. It was made up to 1 L of distilled water and inoculated at 28°C for 9 days.

Figure 1. Fruiting body of *Ganoderma applanatum* collected from Albaha area, Saudi Arabia.

Preservation of fungal strains

For maintenance, *G. applanatum* mycelia was collected from a well grown culture and inoculated in potato dextrose agar slants (Peeled Potato, 250 g; Dextrose, 20 g made up to 1 L with distilled water). Monthly subcultures were made on PDA agar slants with dextrose as carbon source.

Media effect on EPS-producers

A screening experiment was carried out using 5 liquid media in order to determine the most suitable medium for EPS production; [Potato dextrose media (PDA), Malt dextrose media (M). Glucose yeast extract peptone media (GYP) as designed by Wang et al. (2001) consists glucose (40 gm), yeast extract (10 gm) and peptone (5 gm). It is made up to 1 Liter in distilled water. Czapek's media consists of glucose 20 gm, $NaNO_3$ 2 gm, KH_2PO_4 1 gm, MgSO4. $5H_20$ 0.5gm, KCl 0.5 gm and $FeSO_4$. $7H_2O$ 0.001 gm. Both static and shaken cultures were tried in order to select the best for EPS production.

Culture type

Shaken culture

Erlenmeyer flasks (250 ml) were filled with 50 ml of 5 types of media which were Potato dextrose media (PDA), Malt dextrose media (M), Glucose yeast extract peptone media (GYP), and Czapek's media.

The media was inoculated with the fungal disc by using sterilized cork borer of 1 cm diameter. It was obtained from solid parent fresh culture of tested fungi subcultured on solid PDA. The fungal strain was *G. applanatum*. The cultivated flasks with initial pH 6.2 were then incubated at 25°C under shaking condition in a rotary shaker (150 rpm) for 5 days for all tested media (Yun et al., 2002a).

Table 1. Effect of different media on exopolysaccharide production and mycelial growth in *Ganoderma applanatum* under shaken conditions at (150 rpm).

Media	Mycelial drywt g/50 ml	EPS drywt mg/ml	Final pH
Czapek's medium	0.1532±0.01	0.096±0.013	5.8±0.05
Potato dextrose medium.	0.299±0.001	0.196±0.02	5.9±0.1
Malt medium	0.1189±0.008	0.116±0.02	6.1±0.1
Glucose yeast extract peptone	0.4662±0.057	0.320±0.01	5±0.1

At the end of incubation period, EPS was precipitated from the culture filtrate, then lyophilized and its weight was estimated. Also, the dry weight of mycelium was measured after repeated washing of the mycelial pellet with distilled water and drying at 70^{o}C for overnight to a constant weight.

Static culture

The same previous procedure was carried out without shaking in rotary shaker and incubated for 15 days.

Isolation and purification of *Ganoderma applanatum* exopolysaccharides

Ethanol precipitation

The culture filtrate from shaken flasks was centrifuged at 10000 g for 20 min and the resulting supernatant was filtered (0.45 μm, Millipore). The resulting culture filtrate was mixed with 4 times the volume of absolute ethanol, stirred vigorously and kept overnight at 4^{o}C. The precipitated EPS was centrifuged at 10000 g for 20 min discarding the supernatant (Bae et al., 2000). Details for EPS purification and chemical structure will be presented elsewhere.

Rheology of EPS

The theological behavior of different EPS-treatments was studied using cup and Bob viscometer Brookfield DV-III Rheometer, Rheocale 1.4 software, using USA spindle: ULA, model: RV). The sample was sheared in the space between the outer wall of the bob and the inner wall of the cup into which the bob fits. The rotation ranged between 100-250 rpm. The rheograms and viscosity curves were platted using the supplied software. This program needed to be supplied with two readings namely upper and lower ones of torque and rotation (rpm) to detect the shear thinning index (ST). The EPS behavior was deduced according to (ST) values as follows: "1" means that the system behaves as newtonian, lower than "1" indicates that the system is dilatant and more than "1" means that the system is pseudoplastic (Martin et al., 1983).

The following formulas were used to calculate and display the Rheometer data after each packet of data was obtained from the DV-III.

Viscosity (cP) = 100 x TK x SMC x Torque RPM
Shear Rate (1/Sec) = RPM x SRC
Shear Stress (Dynes/Cm2) = TK x SMC x SRC x Torque

Where:

RPM = Current Rheometer spindle speed in RPM
TK = Model spring constant
SMC = Current spindle multiplier constant
SRC = Current spindle shear rate
Torque = Current Rheometer torque in percent expressed as a number between 0 and 100.
*TK, SMC and SRC are obtaining from standard known tables.

Viscosity-concentration relationships

The viscosity of purified EPS at concentrations of 0.25, 0.5, 1.5 and 2.5% was determined at a constant temperature of 25°C.

The effect of different levels of pH on viscosity of EPS

The effect of different pH values at 4.5, 5, 5.5 6.0 and 6.5 on EPS at concentration of 0.25% (W/V) was tested by measuring the viscosity at constant temperature of 25°C (Bae et al., 2000).

Why is pH measured against 0.25% EPS of *G. applanatum*? Because, it has been mentioned by many researchers that pH influences the stability of foams with protein–polysaccharide complexes at their interfaces and plays a vital role in ionization process of solutions, so it is kept as minimum as possible.

Effect of different carbon sources on viscosity

The effect of different carbon sources (arabinose, galactose, glucose, sucrose and carboxymethyl cellulose) on the viscosity of EPS at constant concentration of 0.25% (W/V) was tested by measuring the viscosity at constant temperature of 25°C (Yun et al., 2002b).

RESULTS

Glucose yeast extract peptone media (GYP) proved to be the more suitable for organism growth and exopolysaccharides production which recorded 0.320 + 0.01 and 0.1+0.01 dry weight mg/ml under shaken and static conditions respectively (Tables 1 and 2).

Rheological characterization of EPS

Effect of EPS concentration on its rheology (flow behavior)

The rheological characters of different concentrations of EPS (0.25, 0.5, 1.5 and 2.5%) (w/v) in distilled water were studied using a brook field digital DV III rheometer linked to a computer for processing, recording and storing of data.

Table 2. Effect of different media on exopolysaccharide production and mycelial growth in *Ganoderma applanatum* under static conditions.

Media	Mycelial dry wt. g/50 ml	EPS dry wt. mg/ml	Final pH
Czapek's medium	0.584±0.04	0.064±0.001	5.8±0.05
Potato dextrose medium.	0.595±0.09	0.082±0.01	5.9±0.1
Malt medium	0.386±0.02	0.05±0.005	6.1±0.1
Glucose yeast extract peptone	1.183±0.2	0.12±0.01	5±0.1

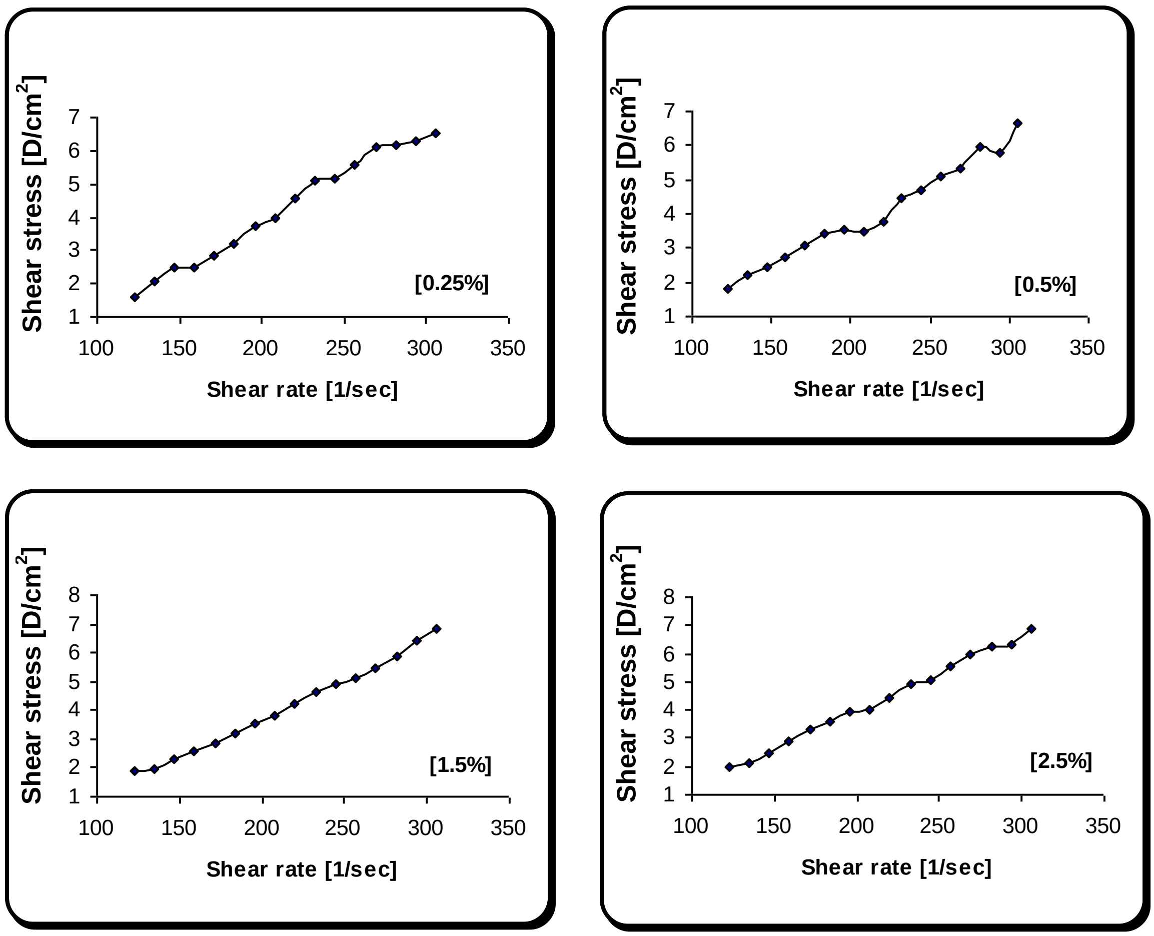

Figure 2a. Shear rate-shear stress relationships.

The rheogram and viscosity curves of different EPS concentrations in distilled water were plotted using Rheocale 1.4 software computer program. The rheograms are presented in Figure 2a and b. It can be noticed that the increment of shear rates (S.R) was accompanied with an increase in the shear stress (S.S), indicating all concentrations of EPS followed dillatent behavior. For further confirmation of these results, the values of ST index (shear thinning index) as well as the solution behavior were determined. This program (Rheocale 1.4 software program) needs to be supplied with two readings namely upper and lower ones deduced from the computer program according to ST values as follows: "1" means that system behaves as Newtonian; less than "1" indicates that the system is dillatent and more than "1" means that the system is pseudo plastic.

This method, which was convenient in identifying the flow behavior at upper and lower shearing rates, was applied for every solution in this work and it was found that the ST index for all solution was less than "1";

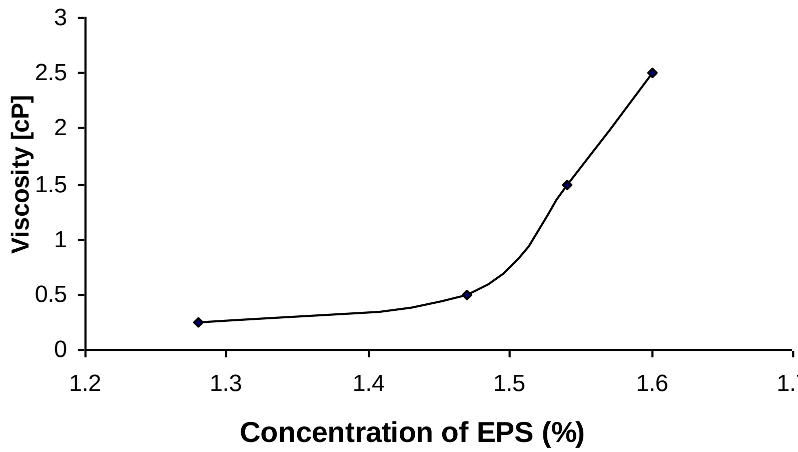

Figure 2b. The flow behavior of EPS from *G. applanatum* at concentration (0.25-0.5%) as computed from Rheocale V.1.4.

Table 3. Detection of ST index of different EPS concentrations.

EPS conc. (w/v)	* RPM	Torque %	** ST Index
0.25	100	2 ± 0.13	0.8 ± 0.04
	110	2.6 ± 0.2	
0.5	100	2.4 ± 0.15	0.87 ± 0.06
	110	2.8 ± 0.20	
1.5	100	2.5 ± 0.10	0.92 ± 0.08
	110	2.9 ± 0.30	
2.5	100	2.7 ± 0.20	0.96 ± 0.09
	110	3.2 ± 0.20	

* ST index is shear thinning index. ** RPM means round per minutes.

Table 4. Relation between EPS concentration and viscosity.

EPS conc. (w/v)	Viscosity (cP)**
0.25	1.30 ± 0.10
0.5	1.47 ± 0.07
1.5	1.54 ± 0.15
2.5	1.60 ± 0.13

* Data at constant (122.3) shear rate. ** cP index is viscosity index.

indicating dillatent behavior of the system (Table 3).

Viscosity

Table 4 Indicates that viscosity of each EPS concentration increased with increasing S.R values which confirm the dillatent behavior of the different concentrations of EPS. It is clear that, the viscosity of EPS increased with increased EPS concentrations.

Effect of different level of pH on the rheology of EPS

The effect of different level of pH on the flow behavior of EPS was studied using Rheocale 1.4 software computer program. The parameters of the previous experiment were studied and the results are illustrated in Table 5. It was found that the viscosity increased with increasing S.R values until reaching pH value 5.5 after which it decreased. So, pH value affects the rheological pattern of EPS. Concerning the viscosity of EPS, pH=5.5 increased the viscosity of EPS solutions as illustrated in Table 6.

Effect of different carbon sources on the rheology EPS

The results of the current experiment are shown in Table 7. It was noticed that different carbon sources affect the rheology of EPS and all solutions had dillatent behavior.

Addition of sucrose increased the viscosity of EPS solutions (Table 8 and Figure 3).

As summarized in Table 8, it could be concluded that

Table 5. Detection of ST index of different level of pH at constant concentrations of EPS at 0.25% (%, w/v).

pH values	*RPM	Torque %	** ST Index
4.5	100	2.0 ± 0.1	0.74 ± 0.06
	125	2.8 ± 0.2	
5	100	2.1 ± 0. 2	0.84 ± 0.07
	125	3.1 ± 0. 1	
5.5	100	2.4 ± 0.2	0.95 ± 0.09
	125	3.2 ± 0.3	
6	100	1.15 ± 0.1	0.72 ± 0.06
	125	1.43 ± 0.2	
6.5	100	1.09 ± 0.1	0.62 ± 0.05
	125	1.42 ± 0.1	

Table 6. Effect of different pH values on viscosity of EPS at constant concentrations of EPS 0.25% (%, w/v).

pH values	* Viscosity (cP)
4.5	1.22 ± 0.10
5	1.34 ± 0.09
5.5	1.54 ± 0.14
6	1.20 ± 0.11
6.5	1.00 ± 0.10

Data at constant (122.3 1/Sec.).

Table 7. Detection of ST index of different carbon sources at constant concentrations of EPS at 0.25% (%, w/v).

Carbon sources (%, w/v)	*RPM	Torque %	** ST Index	*** cP index
Arabinose	100	2.4 ± 0.20	0.74 ± 0.06	0.17 ± 0.01
	110	2.5 ± 0.15		
Galactose	100	2.2 ± 0.20	0.94 ± 0.08	0.13 ± 0.01
	110	2.5 ± 0.16		
Carboxy methyl cellulose (CM)	100	2.4 ± 0.21	0.88 ± 0.07	0.18 ± 0.01
	110	3.0 ± 0.25		
Glucose	100	2.3 ± 0.14	0.97 ± 0.08	0.22 ± 0.02
	110	2.7 ± 0.25		
Sucrose	100	2.3 ± 0.20	0.82 ± 0.07	0.26 ± .016
	110	3.1 ± 0.30		

*** cP index is viscosity index.

sucrose addition enhanced the viscosity of EPS solutions, while arabinose addition decreased EPS viscosity.

DISCUSSION

Exopolysaccharide and endo polysaccharide produced by different fungi, bacteria, algae and endopolysaccharides are found also in plants. Their importance came from their applications as anti-inflammatory and immunoactive (Lull, 2005). Also, as antifibrotic agent on liver fibrosis in rats (Nan et al., 2001). Enopdysaccharides also have a hypoglycemic activity (Kiho, et al., 1999) and exopolysaccharides have

Table 8. Effect of different C. sources on viscosity of EPS at constant concentrations of EPS 0.25% (%, w/v).

Carbon sources 0.25 (%, w/v)	*Viscosity (CP)
Arabinose	1.27 ± 0.1
Galactose	1.44 ± 0.13
Carboxy methyl cellulose	1.40 ± 0.13
Glucose	1.41 ± 0.1
Sucrose	1.47 ± 0.10

*Data at constant (122.3 1/sec.).

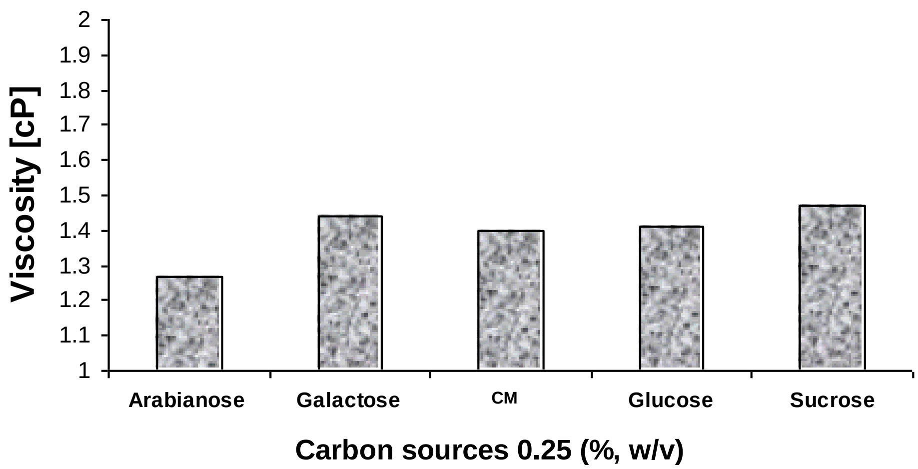

Figure 3. Effect of different C. sources on viscosity of EPS at constant concentrations of EPS 0.25% (%, w/v), (CM): Carboxy methyl cellulose.

a hypoglycemic activity (Jeong et al., 2001). Exopolysaccharides have a protective effect from experimental infections (Nagumo, 1973). They are important as thickening agents (Clementi et al., 1998*)*.

The obtained data indicate that, the shaken culture was favorable for maximum EPS production more than static culture. Our current data indicate that glucose yeast extract peptone media (GYP) is the suitable medium for organism growth and exopolysaccharides production which recorded 0.320 + 0.01 and 0.1+0.01 dry weight mg/ml under-shaken and static conditions respectively (Tables 1 and 2). Osman et al. (2014) have found that yeast extract and peptone led to high production of EPS and mycelial biomass. However, Zapata et al. (2012) studied the effect of different non-conventional carbon sources on mycelial biomass and polysaccharides production in the submerged culture of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum*, in less time, using non-conventional carbon sources to minimize the high costs of current culture media. The optimal medium composition was defined as (g/L): 50 of barley flour, 0.2 of KH_2PO_4, 0.1 of $MgSO_4.7H_2O$, and 1 NH_4Cl. Cultivated under this complex culture medium, the mycelial biomass production was 23.49 ± 0.37 g/L; the extracellular polysaccharides production was 2.72 ± 0.11 g/L; the intracellular polysaccharides production was 2.22 ± 0.06 g/L. Fraga et al. (2014) have stated that a low peptone level (1.65 g L^{-1}) favored mycelium biomass, EPS purity, but a higher supply of peptone (4.80 g L^{-1}) is needed for maximum EPS production. Concerning the carbon source, Shen et al. (2013) reported that xylose is the suitable carbon source for maximum production of EPS from *P.pulmonarius.*

This is almost similar to that concluded by Kim et al. (2003a) who studied the effect of aeration on the production of mycelial biomass and exopolysaccharides in fungus *Paecilamycess sinclairii*. They concluded that aeration is associated with higher hyphal density and increased EPS.

Due to the importance of polysaccharide, a lot off researchers employed their work to study the production of polysaccharides for their medical importance (Mizuno, 1996). Also polysaccharides have many industrial importance like those of *Cordyceps militaris* C738 and *Paecilomyces japonica* (Sinha et al., 2001).

In the present investigation, the effects of different media under shaken conditions at velocity (150 rpm) on exopolysacchrides production and mycelial growth were studied and also under static conditions. The results for shaken conditions confirmed the maximum yield of exopolysaccharide (0.316 mg/ml) for *Ganoderma applanatum* on GYP medium for five days, but was less under static conditions; the EPS was 0.1 mg/ml on GYP media static conditions. These results were similar with those obtained from Martinez et al. (1996) who found that the exopalysaccharid produced from *Hericium erinaceus* and *Hericium laciniatum*d yield more EPS on GYP medium in shaken condition (100 rpm) at 25°C for 25 days.Yun et al. (2002a) studied the EPS production using different media (MCM=mushroom complete medium, Ym= yeast extract malt medium and PMP= malt extract potato dextrose broth medium) to grow different edible mushrooms *Agrocybe cylindracea*, *Ganoderma lucidum* No. 1., *Lentinus edodes*, *Pleurotus ostreatus* No. 1*, P. ostreatus* No. 2 and *P. sajor-caju*. From their result, the EPS production (1158 mgL^{-1}) was achieved in *G. lucidum* under shaken conditions (150 rpm) on MCM medium more than YM medium. The results of mycelial growth of various mushrooms were in the order Cordyceps militaris > *P. ostreatus* No. 1 > *P. trametes*.

In the present study, the rheological property of EPS was described whereas, factors affecting the viscosity such as concentrations of EPS solutions pH level and carbon sources were studied.

The flow property of different concentrations of experimental *G. applanatum* indicated that the system had a dillatent behavior, that is the viscosity of the system increased by increasing shearing rate.

In the present work, shear thinning index measurement was done using Rheocale 1.4 software program at two readings namely high and low ones of torque and rotation (rpm). The experimental EPS of *G. applanatum* has also shear thinning that is, ST index <1 because its viscosity increased with increasing shear rate. This confirms the dilatants behavior of EPS.

So, with increasing EPS concentrations the viscosity increased and EPS increased due to increasing biomass. Relatively few studies have been aimed at quantifying the influence of biomass concentration on rheology. *Penicillium chrysogenum* is a good example in that viscosity is higher when the cell is highly grown (Warren et al., 1995). Yun et al. (2003) concluded that viscosity of EPS of *Cordyceps militaris* was much influenced by EPS conc. than by mycelial biomass. In the present investigation, the effect of different levels of pH on the flow behavior of EPS was studied using Rheocale 1.4 software computer program. It was found that it increased with increased pH value 5.5 after which it decreased. These results are similar to that obtained by Yun et al. 2003), who study the influence of pH on broth rheology and EPS production. The EPS production and viscosity increased at pH value 6. Also, similar results were concluded by Wang and McNeil (1995), who reported that EPS production and EPS viscosity are affected by pH culture.

In present study, it was found from the results that sucrose addition as carbon source enhanced the (consistency index cP) viscosity of EPS solution while arabinose decreased EPS viscosity.

Yun et al. (2002b) studied the influence of carbon sucrose on EPS rheology of *Ganoderma lucidum*. They found that the consistency index (cP) in sucrose medium was markedly higher than that in starch medium while the higher value of flow behavior index (ST) was indicated at the late stationary phase in starch medium. The non-Newtonian behavior (dilatent) is relatively predominant at lower shear rate and low (ST) index (Kim et al., 1983).

Conclusion

In the present study, it was found that glucose yeast extract peptone medium is the most suitable for *G. applanatum* growth and exopolysaccharides production. EPS rheology has a dillatent behavior. The viscosity of each EPS concentration increased with increasing S.R values which confirm the dillatent behavior of different concentrations of EPS. pH value affects the rheological pattern of EPS where pH 5.5 increased the viscosity of EPS. Sucrose addition as carbon source enhanced the (consistency index cP) viscosity of EPS solution while arabinose decreased EPS viscosity. These data give a promising application field of food application and textile industry.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Bae JT, Park, JP, Song CH, Yun JW (2000). Optimization of submerged culture conditions for exo-biopolymer production by *Paecilomyces japonica*. J. Microbiol. Biotechnol. 10:482-487.

Clementi F, Mancini M, Moresi M (1998). Rheology of alginate from *Azotobacter vinelandii* in aqueous dispersions. J. Food Eng. 36(1):51-62.

Fazenda ML, Harvey LM, McNeil B (2010). Effects of dissolved oxygen on fungal morphology and process rheology during fed-batch processing of *Ganoderma lucidum.* J. Microbiol. Biotechnol. 20(4):844-851.

Fraga I, Coutinho J, Bezerra RM, Dias AA, Marques G, Nunes FN (2014). Influence of culture medium growth variables on *Ganoderma lucidum* exopolysaccharides structural features. Carbohydr. Polym. 111:936-946.

Jeong SC, Yang BK, Cho SP, Yun JW, Kim DY, Song CH, Park JB (2001). Production of a hypoglycemic, extracellular polysaccharide from the submerged culture of the mushroom, *Phellinus linteus*. Biotechnol. Lett. 23(7):513-517.

Kiho T, Ookubo K, Usui S, Ukai S, Hirano K (1999). Structural features and hypoglycemic activity of a polysaccharide (CS-F10) from the cultured mycelium of *Cordyceps sinesis*. Biol. Pharm. Bull. 22(9):966-970.

Kim ME, Brown RA, Armstrong RC (1983). The roles of inertia and shear-thinning in flow of an inelastic liquid through an axisymmetric

sudden contraction. J. Non-Newt. Fluid Mech. 13:341-363.
Kim SW, Hwang HJ, Xu CP, Choi JW, Yun JW (2003a). Effect of aeration and agitation on the production of mycelial biomass and exopolysaccharides in an enthomopathogenic fungus *Paecilomyces sinclarii*. Lett. Appl. Microbiol. 36(5):321-326.
Kim SW, Hwang HJ, Xu CP, Sung JM, Choi, JW, Yun JWJ (2003b). Optimization of submerged culture process for the production of mycelial biomass and exo-polysaccharides by *Cordyceps militaris* C738. Appl. Microbiol. 94(1):120-126.
Klupp NL, Chang D, Hawke F, Kiat H, Cao H, Grant SJ, Bensoussan A (2015). *Ganoderma lucidum* mushroom for the treatment of cardiovascular risk factors. Cochrane Database Syst. Rev. 17(2):1-54.
Lamari FN, Kuhn R, Karamanos NK (2003). Derivatization of carbohydrates for chromatographic, electrophoretic and mass spectrometric structure analysis. J. Chromatogr. B 793(1):15-36.
Lull CH, Wichers HJ, Savelkoul HFJ (2005). Antiinflammatory and Immunomodulating Properties of Fungal Metabolites. Mediators Inflamm. 2005 June 9; pp. 63–80.
Martin A, James S, Cammarata A (1983). Physical pharmacy: Physical chemical principles in the pharmaceutical science. Lea and Febiger Philadelphia (U.S.A). pp. 522-542.
Martinez AT, Prieto A, Gutierrez A (1996). Structural characterization of extracellular polysaccharides produced by fungi from the genus *Pleurotus*. Carbohydr. Res. 281:143-154.
Mizuno T (1996). A development of antitumor polysaccharides from Mushroom fungi. J. Foods Ingredients 167:69-84.
Morris VJ (1990) Science, structure and application of microbial polysaccharides. In: gums and stabilisers for the food industry, Edited by G.O. Phillips. Oxford: IRL. pp. 315-328.
Nagumo N (1973). Protective effect of the mushroom polysaccharide *Schizophyllan agaireus* experimental bacterial infections. Jpn J. Antibiot. 26(3):277-283.
Nan JX, Park EJ, Yang BK, Song CH, Ko G, Sohn DH (2001). Antifibrotic effect of extracellular biopolymer from submerged mycelial cultures of cordyceps militaris on liver fibrosis induced by bile duct ligation in rats. Arch. Pharm. Res. 24(4):327-332.
Osman M, Ahmed W, Hussein F, El-Sayed H (2014). Endopolysaccharides production and growth of *Flammulina velutipes* 6 under submerged conditions. Chem. Biol. Phys. Sci. 4(4):3350-3360.
Reshetnikov SV, Wasser SP, Tan KK. (2001). Higher basidiomycetes as source of antitumor and immunostimulating polysaccharide (review). Int. J. Med. Mushrooms 3:361-394.
Shen JW, Shi CW, Xu CP (2013). Exoploysaccharides from *Pleurotus pulmonarius* fermentation, optimization, characterization and antioxidant activity. Food Technol. Biotechnol. 51(4):520-527.
Sinha J, Bae JT, Park JP, Kim KH, Song CH, Yun, JW (2001). Changes in morphology of *Paecilomyces japonica* and their effecton broth rheology during production of exo-biopolymers. Appl. Microbiol Biotechnol. 56:88-92.
Wang JC, Hu SH, Su CH, Lee TM (2001). Antitumor and immunoenhancing activities of polysaccharide from culture Broth of *Hericium* SPP. Kaohsiung. J. Med. Sci. 17:461-467.
Wang YC, McNeil B (1995). pH effects on exopolysaccharide and oxalic acid production in cultures of *Sclerotium glucanicum*. Enzyme Microb. Technol. 17:124-130.
Warren SJ, Keshavar Z, Morre E, Shamlaou PA, Lilly MD, Thomas CR, Dixon, K. (1995). Rheologies and morphologies of three actinomycetes in submerged culture. Biotechnol. Bioeng. 45:80-85.
Xu W, Hunag J, Cheung PCK (2012). Extract of *Pleurotus pulmonarius* suppresses liver cancer development and progression through inhibition of VEGF-induced P13K/AKT signaling pathway. Plos One 7(3):e34406.
Yun JW, Kim SW, Xu CP, Hwang HJ, Choi JW, Kim CW(2003). Production and characterization of exopolysaccharides from an enthompathogenic fungus Cordyceps militaris NG3. Biotechnol. Prog. 19(2):428-435.
Yun JW, Song CH, Cho YJ, Park JP, Hwang HJ, Kim SW (2002a). Mycelial growth and exo-biopolymer production by submerged culture of various edible mushrooms under different media. Lett. Appl. Microbiol. 34:56-61.
Yun JW, Song CH, Kim SW, Hwang HJ, CHO YJ (2002b*).* Effect of carbon source and aeration rate on broth rheology and fungal morphology during pigment production by *Ganoderma lucidum* in a batch bioredctor. J. Biotechnol. 95:13-23.
Zapata P, Rojas D, Atehortúa L (2012). Production of biomass, polysaccharides, and ganoderic acid using non-conventional carbon sources under submerged culture of the Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W.Curt.:Fr.)P. Karst. (higher Basidiomycetes). Int. J. Med. Mushrooms 14(2):197-203.
Zhang L, Peng Y (2003). Characterization of a polysaccharide-protein complex from *Ganoderma Tsugae* mycelium by size-exclusion chromatography combined with laser light scattering. J. Biochem. Biophys. Methods 1679:1-10.