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Partial Enzymatic Hydrolysis of Glucomannan and Its Mathematical Model

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ABSTRACT

The study of enzymatic hydrolysis of glucomannan (GM) was currently limited to obtain low molecular weight glucomannan, and was not specifically studied for spray drying feed applications. This research aimed to investigate the effect of enzyme concentration and duration of enzymatic hydrolysis of GM on the characteristics of hydrolyzed glucomannan (HGM). Moreover, the kinetic models of viscosity reduction in enzymatic hydrolysis of glucomannan were also studied. To achieve the goal, the GM was hydrolyzed using various enzyme concentrations (5 to 20 mg/l) for 300 min. Profiles of viscosity, average molecular weight (Mw), degree of polymerization, and antioxidant activity of HGM were observed. The kinetics of viscosity reduction was modeled with 1st-order kinetics, 2nd-order model, and Mahammad's order. An enzyme concentration of 20 mg/l (1% GM solution) was the fastest to reach the desired viscosity for spray drying feed purposes. The model of n^{th}-order was the best fitted to the viscosity reduction with R^2 equal to 0.9935, so the constants k = 1.1842 (Pa.s n^{-1}.t)$^{-1}$ and n = 0.6328 are obtained. The hydrolysis improved the antioxidant activity of HGM as the enzyme concentration increases. This antioxidant result highlighted the advantage of using HGM for coating and encapsulating the active compound which also offers oxidation protection.

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NOMENCLATURE

[Ez]	Enzyme concentration (mg/l)	k_n	n^{th}-order model constant [(Pa.s n^{-1} .t) $^{-1}$]
DNS	3,5-Dinitrosalicylic acid	k_m	Mahammad model constant (s^{-1})
DP	Degree of polymerization	Mw	Molecular weight (Da)
DPPH	2,2-Diphenyl-1-picrylhydrazyl	RS	Reducing sugar (ppm)
DRS	Drect reducing sugar	R^2	Coefficient of determination
EE	Encapsulation efficiency	t	Time (min)
$Fe_{initially\ added}$	Initial iron weight added to the solution (g)	TS	Total sugar (ppm)
Fe_{sample}	Measured iron content in powder (g)	W	Dried sample weight (g)
GM	Glucomannan	**Greek Symbols**	
HGM	Hydrolyzed glucomannan	α	Mark-Houwink constant
K	Mark-Houwink constant	α_M	Mahammad model constant
k_1	First-order model constant (s^{-1})	η	Viscosity (Pa-s)
k_2	Second-order model constant [(Pa.s.T)$^{-1}$]		

1. INTRODUCTION

Glucomannan (GM) is a neutral polysaccharide consisting of D-mannose and D-glucose bonding by β-1,4 linkage [1] with 5-10% of this polysaccharide is acetyl groups [2]. This amorphous polymer becomes the highest viscosity among various gum types with an average molecular weight (Mw) of 200 to 2000 kDa [3]. One percent of this heteropolysaccharide has apparent viscosity and average Mw up to 10,000 centipoise and 2.7×10^5 Da, respectively. The molecular structure of GM is shown in Figure 1.

As natural polysaccharides, GM is preferable for environmental purposes as it is biodegradable [4]. Its

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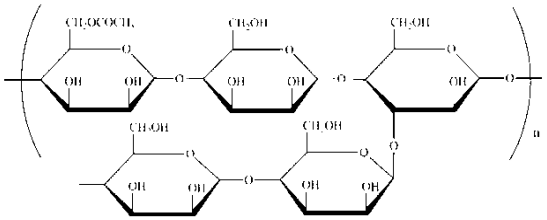


Figure 1. Molecular structure of glucomannan [5]

high viscosity provides a benefit in encapsulation applications by trapping more concentrations of active substances. However, this property leads to a challenge in its application as a matrix in spray drying encapsulation since the feed is required to be flowable [6, 7]. High viscosity prevents the formation of feed atomization in the drying chamber, resulting in drying to be insufficient because of the particle size is too large and even could block the atomizer nozzle. Sosnik and Seremeta [8] reported that approximately 0.3 Pa-s is the maximum viscosity of the feed solution for Büchi Mini Spray Dryer B-290. Hence, the viscosity of GM needs to be adjusted before spray drying application, without neglecting to obtain a high-yield product. Hydrolysis had successfully decreased GM viscosity and produced hydrolyzed glucomannan (HGM) [9, 10]. In addition, GM hydrolysis has been reported to improve the antioxidant activity of HGM [11].

Attempts to reduce average Mw of GM were reported using acids such as H_2SO_4 [12] and citric acid [13]. These processes require special specifications of the equipment to avoid oxidation. In addition, they generate acid waste, and are difficult to control the average Mw of the product. Li et al. [14] conducted a mechanical hydrolysis technique for GM using ultrasound. This method requires specific equipment to produce ultrasonic waves, which is costly for large capacity applications. Using the enzymatic method, hydrolysis offers mild reaction conditions, is easily controlled reaction, and environmentally friendly [15]. Wattanaprasert et al. [6] successfully applied β-mannanase to hydrolyze GM. Unfortunately, this enzyme is not widely available. Cellulase or 4-beta-D-glucan 4-glucanohydrolase is a specific enzyme that hydrolyzes β-1,4-glycosidic bonds of polysaccharides and produces oligosaccharides and/or monosaccharides [16]. Considering β-1,4 glycosidic as the primary linkage of GM, this commonly available enzyme is an excellent candidate to cleavage the linkage. Although this enzyme has been applied in GM hydrolysis for iron and flavour encapsulant [9, 15, 17], the combined effect of enzyme concentration and its kinetic in preparing spray drying feed is still less explored [18].

Hence, this work aims to examine the effect of cellulase concentration and hydrolysis period on the characteristics of HGM, including the viscosity, average Mw, degree of polymerization, and antioxidant capacity. The kinetic of the GM hydrolysis was also studied to predict a suitable pretreatment on GM prior to spray dryer application.

2. MATERIALS AND METHOD

2. 1. Materials The materials used in this study were Now Food® Glucomannan powder, cellulase enzyme (4-beta-D-glucan 4-glucanohydrolase) (EC 3.2.14) from Sigma-Aldrich with 0.3 U/mg from Sigma-Aldrich, and other chemicals obtained from Merck KGaA used without purification.

2. 2. Glucomannan Hydrolysis Visual diagram of glucomannan hydrolysis process is shown in Figure 2. One liter of GM solution (1% w/v) was hydrolyzed with cellulase (5, 10, 15, 20 mg/l) under 350 rpm constant stirring (Hightech Mixer IKA RW 20 Digital) at room temperature. After 300 min, the enzyme was inactivated by heating up the solution at 80°C for 10 min to stop the reaction.

2. 3. Total Sugar and Reducing Sugar Total sugar (TS) was defined as the amount of sugar in the sample, while Direct Reducing Sugar (DRS) was the amount of reducing sugar. The degree of polymerization (DP) was the ratio of TS to DRS as calculated by Equation (1).

$$DP = \frac{TS}{DRS} \quad (1)$$

The method of TS was conducted based on method developed by Nakasaki et al. [19], while the method of DRS was determined using the 3.5-dinitrosalicylic acid (DNS) [20]. Basically, the DNS method was based on reducing DNS in alkaline atmosphere which was identified by the absorbance at 550 nm wavelength.

2. 4. Viscosity and Its Kinetic Model GM or HGM solution (500 ml) was placed in beaker glass for viscosity measurements using spindle number 7 of Brookfield viscometer (Model RVTDV II, Brookfield Engineering Laboratories, Inc., Stoughton, MA) at 28±1°C at 100 rpm. Triple measurements were carried out for each sample.

The rheokinetic model was developed to estimate the reaction rate of the experimental data. Four mathematical models, i.e., order 1 (Equation (2)), order 2 (Equation (3)), order-n (Equation (4)), and Mahammad's model (Equation (5)) [21], were fitted to the viscosity of experimental data. The one with the coefficient of determination (R^2) close to 1 was the fittest model representing the glucomannan hydrolysis.

$$\eta = \eta_o e^{-k_1 \times t} \quad (2)$$

$$\frac{1}{\eta} = \frac{1}{\eta_o} + k_2 \times t \quad (3)$$

$$\eta = (\eta_o^{1-n} - (1-n) \times k_n \times t)^{\frac{1}{(1-n)}} \quad (4)$$

$$\ln(\frac{\eta 0}{\eta}) = \frac{\alpha_M}{3} \times \ln(1 + k_m \times t) \quad (5)$$

2. 5. Average Molecular Weight The intrinsic viscosity of the solutions was conducted using Cannon Fenske Viscometer size 100. Various HGM concentration solutions (0.01, 0.02, 0.03, 0.04, and 0.05 g/l) were prepared for each sample. Average molecular weight was calculated by Mark Houwink using Equation (6), with the specific constant value *K* and *a* was 5.9610 x10^{-4} and 0.7317, respectively [22].

$$\eta = 5.9610 \times 10^{-4} \times Mw^{0.7317} \quad (6)$$

2. 6. Hydroxyl and DPPH Radical Scavenging Activity Determination Hydroxyl radical and DPPH scavenging activity was measured using the method of Ding et al. [23] for HGM solution after 300 min hydrolysis. The sample (1.0 ml) was mixed with 1.0 ml of phosphate buffer (0.4 mM, pH 7.4), 1.0 ml 1.10-phenanthroline hydrate (2.5 mM), 1.0 ml $FeSO_4$ (20.5 mM), and 0.5 ml H_2O_2 (20 mM, 1%, v/v). The mixture was incubated for 60 min at 37°C, the absorbance of the mixture was measured at 536 nm (A_s). In addition, the blank mixture without sample addition also measured for its absorbance (A_0).

Freshly-prepared DPPH in 95% ethanol solution (0.1 mM, 4.0 ml) was incubated with the test sample (1.0 ml). After mixing for 1 min, the solution was incubated for 30 min at room temperature in dark conditions. The absorbance of the solution was read at a wavelength of 517 nm.

The inhibition activity (IA) was calculated using Equation (7), while IC_{50} showed the sample concentration which prevented 50% of total oxidation.

$$IA\ (\%) = \frac{A_0 - A_s}{A_0} \times 100\% \quad (7)$$

3. RESULTS AND DISCUSSIONS

β-mannanase has commonly been applied in GM hydrolysis [24]. However, this enzyme is not broadly available. Cellulase or 4-beta-D-glucan 4-glucanohydrolase, the more commonly found enzyme, is a typical specific enzyme to break β-1,4-glycosidic, the linkage between D-mannose and D-glucose of GM. The effectivity of this enzyme to hydrolyzed GM, specifically to fulfill characteristics for spray dryer feed, was studied at various enzyme concentrations. Degradation of viscosity was modeled subsequently.

3. 1. Viscosity and Average Molecular Weight Figure 3 shows a decrease in HGM viscosity during hydrolysis at all enzyme concentrations. All viscosity of the samples dropped significantly in the first 50 min, followed by a moderate decrease. Indeed, only a slight viscosity change was observed at higher enzyme concentrations after 90 min. Increasing enzyme concentration allowed more contact with GM and cleaved more GM linkages at the same duration reaction. As a result, an insignificant change of the viscosity was observed afterward. All samples achieved 300 cPs in different hydrolysis periods, in which the highest enzymatic concentration reached the viscosity in the shortest time, only 45 min. Meanwhile, the lowest enzyme concentration (5 mg/ml) reached the viscosity target after 300 min hydrolysis. These conditions which allow to reduce GM viscosity to about 300 cPs and fulfill the viscosity requirement for spray dryer feed [8] were recommended for GM pretreatment prior to spray dryer application. A similar trend in viscosity reduction of HGM after enzymatic hydrolysis was also reported by Liu et al. [11]. Using β-mannanase, Bhaturiwala et al. [25] also obtained 320 cPs viscosity of hydrolyzed glucomannan after 24 h incubation time. However, a shorter duration of hydrolysis (<5 h) should be conducted to prevent the growth of other unwanted microorganisms [26].

Viscosity reduction was modeled to predict the HGM viscosity in a certain hydrolysis time. Four mathematical models were fitted to the viscosity reduction. The constants of all models fitting are presented in Table 1.

Most of the model fittings showed R^2 above 0.9 for all enzyme concentrations, except the 2nd order model. Model of nth order was superior to describe viscosity decrease of all enzyme concentrations than other models

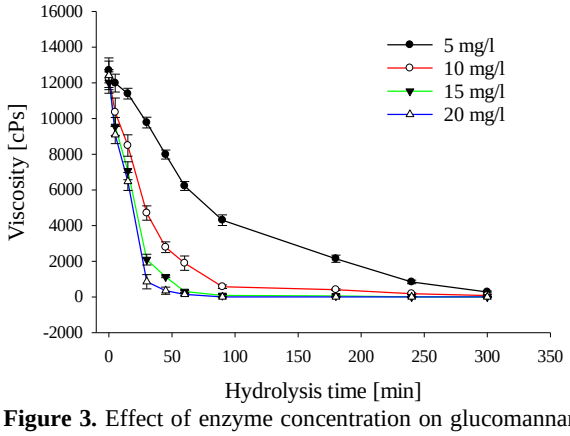


Figure 3. Effect of enzyme concentration on glucomannan viscosity

TABLE 1. The constants of the kinetics models in decreasing profile of the viscosity of HGM at various enzyme concentrations

Constants	[Ez] = 5 mg/l	[Ez] = 10 mg/l	[Ez] = 15 mg/l	[Ez] = 20 mg/l
1st order				
k_1 [s^{-1}]	0.0108	0.0307	0.0474	0.0595
R^2	0.9933	0.9951	0.9878	0.9806
2nd order				
k_2 [$(Pa.s.T)^{-1}$]	0.0000	0.0000	0.0028	0.0038
R^2	0.6775	0.7859	0.6973	0.8801
nth order				
k_n [$(Pa.s\ n^{-1}.t)^{-1}$]	0.0191	0.1341	1.1842	22.6446
n	0.9364	0.8329	0.6328	0.3268
R^2	0.9936	0.9960	0.9935	0.9910
Mahammad model				
α_M	9.0800	8.0700	8.0699	10.0899
k_m [s^{-1}]	0.0061	0.0168	0.0608	0.0599
R^2	0.9380	0.9786	0.8876	0.9398

in, in which the lowest R^2 is 0.9910. Plot of all models to viscosity decrease of HGM using 15 ppm cellulase is presented in Figure 4. This fitting profile supported the R^2 values of TABLE 1. The model of 2nd order was the worst with R^2=0.697, while nth order (R^2=0.993) showed the best fit one. The goodness of nth order suggested that the model is suitable to predict the viscosity profile during GM hydrolysis under various enzyme concentrations.

The relationship between viscosity and average Mw is determined based on the Mark-Houwink equation. The increase in viscosity positively correlates with average Mw [27]. The work of cellulase in cleaving β,1-4 linkage led to average Mw reduction of HGM, as shown in Table 2. Since all samples have similar viscosity after 300 min hydrolysis (Figure 3), the average Mw of all samples showed similar values ranging from 83.96-85.68% reduction compared to the initial average Mw of GM. Bhaturiwala et al. [25] found a similar value of average Mw decrease after glucomannan hydrolysis using β-mannanase, which had ~86.5% reduction after 24 h of hydrolysis.

3. 2. Reducing Sugar and Degree of Polymerization Viscosity modifications were parallel with the change of DRS and DP (Figure 5). During GM hydrolysis, oligosaccharides and reducing sugars were released concerning the enzyme work. The high enzyme concentration provided more active sites to cleave the GM linkages, resulting in more reducing sugars. The highest enzyme concentration (20 mg/l) sharply increased DRS from 1.2 to 78.1 mg/g sample in the first 30 min, followed by an insignificant change of reducing sugar afterward and reached 85.1 mg/g sample after 300 min. Meanwhile, the lowest enzyme (5 mg/l) produced 78.81 mg/g sample after 300 min hydrolysis and released the lowest reducing sugar concentration than other enzyme concentrations even after 300 min.

TABLE 2. Effect of enzyme concentration on average molecular weight (Mw) of HGM after 300 min hydrolysis

Enzyme concentrations (mg/l)	Average molecular weight		Percentage of decrease (%)
	Initial GM (Da)	HGM (Da)	
5	751,628	120,550	83.96
10		118,564	84.23
15		112,190	85.07
20		107,611	85.68

Hydrolysis did not have an impact on the TS of HGM. Hence DP of samples was only influenced by DRS. As DP is a ratio between TS and DRS, an increase of DRS led to an opposite value of DP. DP HGM decreased from 7.64 to 1.67 within 15 min hydrolysis using 20 mg/l. The lowest concentration required 180 min to obtain a similar DP value. Jian et al. [28] produced 2-9 DP values of HGM in 10 h after a treatment combination of radiation and endo-1, 4-β-D-glucanase under incubator condition. Our study needed a shorter time to reach the range DP than that of Jian et al. [28] due to differences in the hydrolysis method. Our hydrolysis was conducted under continuous stirring, allowing more intensive collision between the enzyme and substrate, which reduced the reaction time.

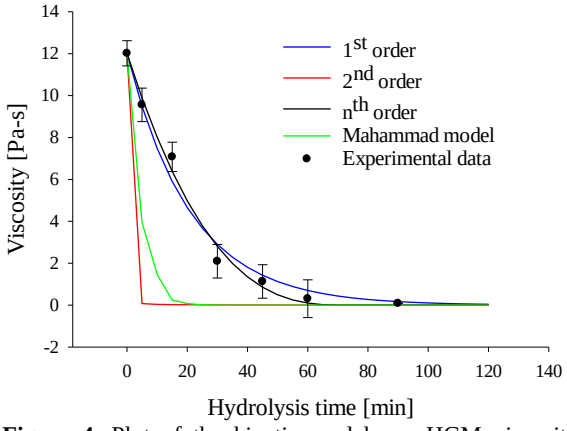
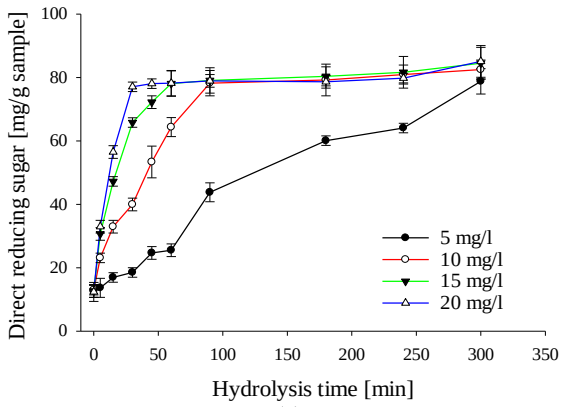


Figure 4. Plot of the kinetic models on HGM viscosity reduction using 15 ppm cellulase



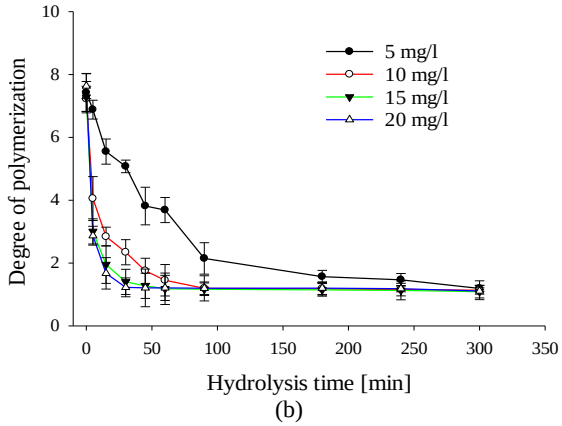
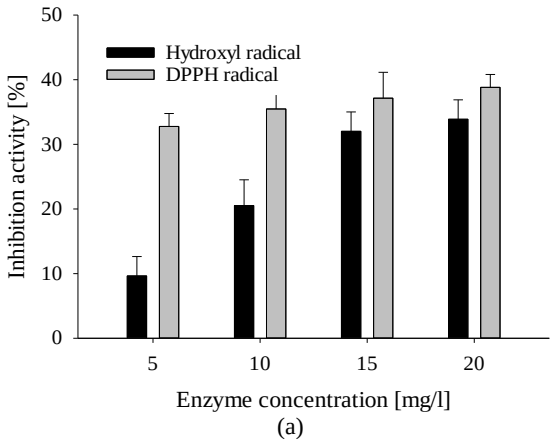


Figure 5. Effect of enzyme concentration on (a) direct reducing sugar and (b) degree of polymerization of HGM



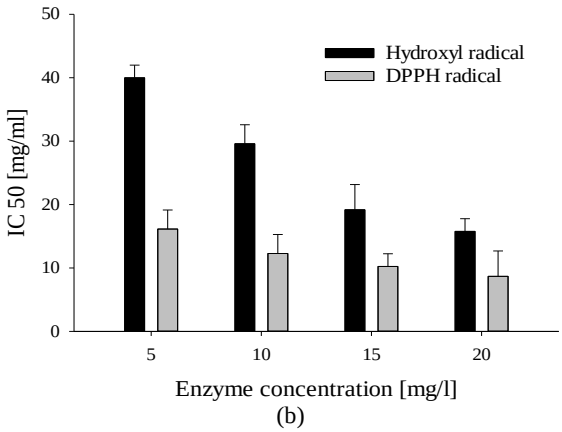


Figure 6. Effect of enzyme concentration on (a) HGM inhibitory activity and (b) IC_{50} HGM

3. 3. Antioxidant Activity Encapsulation using the spray drying method involves high-temperature treatment to obtain final dry powders containing active substances. The use of a matrix that can protect the active compounds from heat degradation helped suppress oxidation during the mixing of ingredients and the spray drying process. Antioxidant activities of HGM after 300 min hydrolysis using various enzyme concentrations are presented in Figure 6. The activities of HGM were presented as inhibition activity toward DPPH radical and hydroxyl radical. In both antioxidant determinations, scavenging activities of radicals increased with enzyme concentration. Higher enzyme concentration led to produce more numbers of shorter HGM molecules. The DRS of HGM ranged 78.81±2.01–85.10±4.98 mg/g sample, in line with enzyme concentration. Wardhani et al. [29]and Tripetch et al. [30] reported an increase in antioxidant activity after GM hydrolysis. A similar effect of enzyme concentration on the inhibition activity of HGM was reported by Liu et al. [11] using β-mannanase. The study of Cui et al. [24] confirmed that converting polysaccharides to oligosaccharides supports the increase of antioxidant activity.

HGM performed different sensitivity to respond to the inhibition activity and IC_{50} of DPPH and hydroxyl radicals. HGM showed stronger as the DPPH radical scavenger than as the hydroxyl radicals. HGM allows more significant inhibition of DPPH than hydroxyl radical [11]. Interestingly, the HGM hydroxyl activity rose triple from 9.63±3.12% to 33.88±3.01% while DPPH scavenging inhibition was slightly improved from 32.77±1.99% to 38.82±2.11% under 5 to 20 mg/l enzyme treatment at 300 min. The slight difference in responses between the two antioxidant activity methods could be due to different mechanisms of the methods. DPPH proton-free radicals will be reduced when exposed to antioxidants that act as proton radical acceptors. Hence, the DPPH assay is based on the ability of an antioxidant compound to reduce an odd electron of the nitrogen atom in DPPH by receiving a hydrogen atom to the corresponding hydrazine [31]. Meanwhile, hydroxyl radical as the most reactive free radical could form hydrogen peroxide in the presence of metal ions such as iron [32, 33].

Apart from oligosaccharides, some researchers reported the contribution of phenolics compounds on supporting antioxidant properties of polysaccharides [34-36]. Hu et al. [37] reported a GM native of 98% purity contains 0.26% total phenolic and 0.97% total protein which both contribute to the scavenging activities against hydroxyl. In natural sources, most phenolics exist in a form bound to sugars, proteins, or lipids. Releasing total phenolic content and increasing DPPH scavenging activity on soybeans through fermentation was reported by Wardhani et al. [38]. Alrahmany et al. [39] succeeded in releasing the phenolics in rice bran and oat using cellulase, which improved the total phenolic and antioxidant activity. Moreover, some reports also demonstrated a relationship between the structures of the phenolic and their efficacy on antioxidant activity [40]. We argued that a similar phenomenon occurred in the HGM of this study. The more enzymes involved in the hydrolysis, the more phenolic compounds are released which increases the ability of HGM to scavenge the radicals. Since reports on exploring the relationship between enzymatic hydrolysis of GM and their phenolic antioxidant activity are still rarely available, therefore, an integrated study on this topic needs to be conducted in the future. Overall, this antioxidant result highlighted the advantage of using HGM for coating and encapsulating the active compound which also offers oxidation protection.

4. CONCLUSION

In general, viscosity, DP, and average Mw of HGM decreased over the hydrolysis treatment. Conversely, the hydrolysis increased the antioxidant activity of HGM which showed its potential in protecting bioactive from oxidation. Hydrolysis of 1% GM using 20 mg/l cellulase allowed to reach 300 cPs in a shorter time of hydrolysis, hence, fulfilling the viscosity of HGM for spray dryer feed. The reduction viscosity of GM during hydrolysis was best represented by n^{th} order ($R^2 \geq 0.991$).

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Persian Abstract

چکیده

مطالعه هیدرولیز آنزیمی گلوکومانان (GM) در حال حاضر محدود به تلاش برای به دست آوردن گلوکومانان با وزن مولکولی کم بود و به طور خاص برای کاربردهای خوراک خشک کردن اسپری مورد مطالعه قرار نگرفت .این تحقیق با هدف بررسی اثر غلظت آنزیم و مدت زمان هیدرولیز آنزیمی GM بر ویژگی‌های گلوکومانان هیدرولیز شده (HGM) انجام شد .علاوه بر این، مدل‌های جنبشی کاهش ویسکوزیته در هیدرولیز آنزیمی گلوکومانان نیز مورد مطالعه قرار گرفت .برای دستیابی به هدف، GM با استفاده از غلظت های مختلف آنزیم 5) تا 20 میلی گرم در لیتر (به مدت 300 دقیقه هیدرولیز شد .مشخصات ویسکوزیته، میانگین وزن مولکولی(Mw) ، درجه پلیمریزاسیون و فعالیت آنتی اکسیدانی HGM مشاهده شد .سینتیک کاهش ویسکوزیته با سینتیک مرتبه1، مدل مرتبه 2 و دستور محمد مدل‌سازی شد .غلظت آنزیم 20 میلی گرم در لیتر محلول1 ٪ (GM) سریع ترین برای رسیدن به ویسکوزیته مورد نظر برای اهداف خوراک خشک کردن اسپری بود .مدل مرتبه n بهترین برازش را برای کاهش ویسکوزیته با R2برابر با 0.9935 داشت، بنابراین ثابت‌های k = 1.1842 (Pa.s n-1.t)-1 و n = 0.6328 به دست می‌آیند .هیدرولیز فعالیت آنتی اکسیدانی HGM را با افزایش غلظت آنزیم بهبود بخشید .این نتیجه آنتی اکسیدانی مزیت استفاده از HGM را برای پوشش و محصور کردن ترکیب فعال که همچنین محافظت در برابر اکسیداسیون را ارائه می دهد برجسته کرد..