Facile depolymerization process of β-glucan through the use of a high pressure homogenizer

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Abstract

High Molecular weight β-glucan was depolymerized using a high pressure homogenizer with a micro orifice module. 5%(w/v) β-glucan solution was prepared with 1% (w/v) sodium hydroxide solution. The β-glucan solutions were treated through a high pressure homogenizer at 500, 1000 and 1500 bar for 1 to 5 cycles. The pressure and the number of passes increase the make viscosity and molecular weight decrease without the use of any additives such as strong acid/alkali solutions. Therefore, the high pressure homogenization process could be used in commercial processes as an effective method to resolve the physical problems involved in the use of β-glucan with high viscosity and low solubility. The depolymerized β-glucan was characterized by viscometer, GPC, FT-IR, UV-Vis and XRD.

Keywords: β-glucan, Depolymerization, High pressure homogenizer, Micro orifice module

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1. Introduction

The natural sources of β-glucan are bacteria, yeast, algae, mushrooms, barley as well as oats. The chemical structure of β-glucan depends on the source it is isolated from. β-glucan is a long-chain, multidimensional polymer of glucose, in which particular particles of glucopyranose are linked with β type glycosidic bonds, linearly, in a (1→3) and/or (1→4) structure or in a branched way, i.e. with side chains of varying lengths, linked to the main core with β-(1→6) type glycosidic bonds. In addition, β-glucan is a biocompatible, biodegradable and non-toxic polymer, which makes it attractive for applications in the medical, pharmaceutical, cosmetic and food industries. However, the high molecular weight β-glucan shows poor solubility in neutral pH aqueous solutions and high viscosity in solution, which limits its applications [1-5].

β-glucan is depolymerized to obtain low molecular weight β-glucan. Natural polymers with a polysaccharide structure can be depolymerized by acidic hydrolysis or enzymatic treatment. The acid hydrolysis process is often time- and energy consuming, involves use of chemicals and generates waste. Moreover, being a multi-parameter process, it is not always easy to control, although considerable progress in this technique has been achieved. Enzymatic processing is a relatively complex procedure and expensive [6-8].

Depolymerization with a high pressure homogenizer seems to be a promising alternative. It is usually performed in aqueous solution, although suspensions can also be treated. Figure 1 shows a schematic diagram of the reaction chamber with a micro orifice (Z-type). The fluid passes an orifice module under ultra high pressure, and supersonic speeds result due to a rapid decrease in pressure. During the high pressure homogenization, the fluid is affected by high energy processes such as impacts, cavitation and shear forces due to turbulence. Therefore, the high energy of the high pressure homogenizer can lead to depolymerization because of the intense mechanical and chemical effects associated with shear, cavitation, and impact effects [9-12].

This work reports the effect of depolymerization using a high pressure homogenizer through the control of pressure and the number of passes. In addition, the depolymerized β-glucan was characterized by viscometry, gel permeation chromatography (GPC), Fourier-transform infrared spectroscopy (FT-IR), and UV-Vis spectroscopy, X-ray diffraction (XRD).

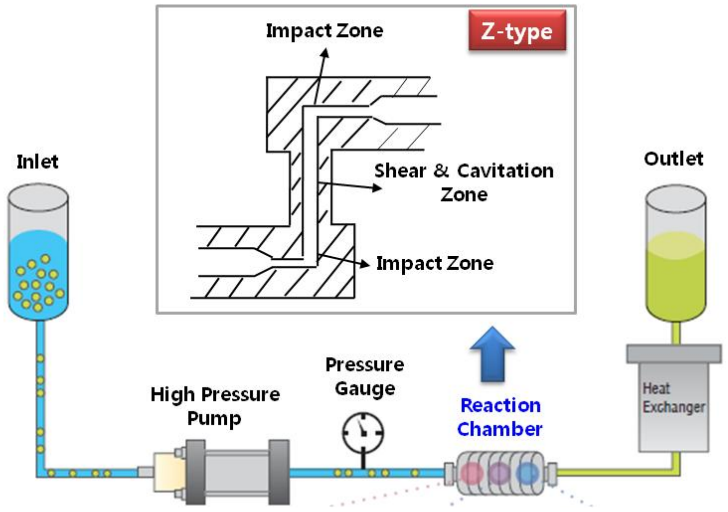

Figure 1. Schematic of the high pressure homogenizer with reaction chamber.

2. Experimental

2.1. Materials

β-glucan supplied by Wako Chemical Reagent Co., Ltd. Sodium hydroxide (NaOH) and Acetic acid (CH_3COOH) were purchased from Samchun. All chemicals used were reagent grade.

2.2. Preparation of Depolymerized β-glucan

5%(w/v) β-glucan solution was prepared with 1%(v/v) sodium hydroxide solution. The β-glucan solutions were then passed through a high pressure homogenizer (Nano Disperser NLM-100, ILSHIN AUTOCLAVE) with a micro orifice module operated at 500, 1000 and 1500 bar for 1 to 5 homogenization cycles. A micro orifice module with a 75 ㎛ inside diameter (Z type) was used in the high pressure homogenizer. The chitosan solution after high pressure homogenization was precipitated with 1%(v/v) acetic acid to pH 6.0. The precipitated β-glucan was recovered by centrifugation, washed several times with deionized water, and freeze dried.

2.3. Characterization of Depolymerized β-glucan

The viscosity of the β-glucan solution was measured using an AND company SV-10 viscometer in a constant-temperature water bath at 25 ± 0.5 ℃ as the mean of three replicates from the same chitosan solution. GPC was used for the qualitative evaluation of the reduction in molecular weight of β-glucan on an Agilent 1100 GPC instrument equipped with a 2 × Plaqagel-OH mixed (7.8 × 300 mm) column and refractive index detector. UV-Vis spectra were taken on a Shimadzu UV-1601 PC in the range of 200 to 500 nm. The XRD patterns of the depolymerized β-glucan were obtained on a Rigaku diffractometer using Cu Kα radiation operating at 40 kV and 50 mA with a scanning rate of 2 ° min^{-1} from 5 to 40 °. The FT-IR spectra were measured on a Thermo Nicolet 6700 spectrophotometer by the potassium bromide pellet method in the range of 400 - 4000 cm^{-1}.

3. Results and Discussion

3.1. Viscosity of the β-glucan solution

The viscosity of the β-glucan solution during the high pressure homogenization is presented in Figure 2. As the pressure and the number of passes increased, the β-glucan solution showed a significant decrease in viscosity. In particular, the viscosity of the β-glucan solution under pressure of 1000 and 1500 bar was able to be decreased sharply by increasing the number of passes from 1 to 5 passes. The viscosity of the original β-glucan solution was 1190.7 cp, whereas the viscosity of the 5 passes sample at 1500 bar was 190.1 cp, with a decrease rate of about 84.0% compared with the original β-glucan solution. The decrease in the viscosity occured during the high pressure homogenization of the β-glucan solution due to the high energy shear, impact and cavitation effects. The decrease in viscosity has been reported to be due to depolymerization which occurred through the breakage of the glycosidic bonds of the β-glucan [6,8,13].

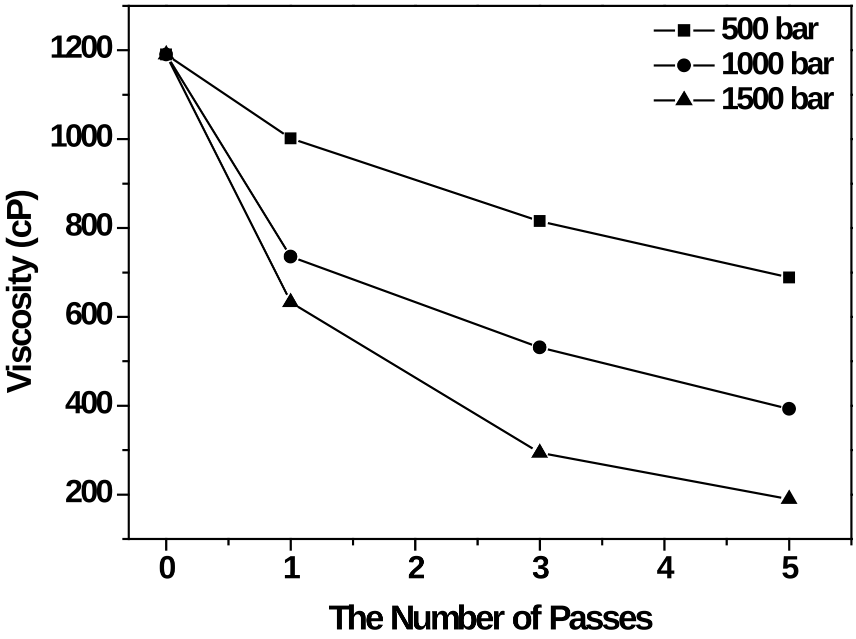

Figure 2. Effect of homogenization pressure and number of passes on the viscosity of β-glucan solution.

3.2. Molecular weight of β-glucan

The average molecular weight was analyzed by GPC to determine the molecular weight of β-glucan after high pressure homogenization. The average molecular weight of the β-glucan by high pressure homogenization is shown in Figure 3. When the original β-glucan solution was high pressure homogenized at 1500 bar, the corresponding molecular weight of chitosan decreased from 174 to 27 kDa. The percentage decrease in the molecular weight was 84.4%. As the number of passes increased, the β-glucan showed a significant decrease in molecular weight. The changes that occur after high pressure homogenization might be due to the breakage of the glycosidic bond of the polysaccharide [6,8]. This appears to be in good agreement with the data on the viscosity of the β-glucan solution. In addition, this result is well supported by the reports of depolymerization by the high pressure homogenization of polysaccharides such as chitosan [14]. We speculate that the decrease in the molecular weight occurred due to the high energy of the high pressure homogenizer in the form of shear, cavitation and impact effects. The entanglement and stretch of the β-glucan polymer chain was disentangled by shear force. In addition, the high molecular weight β-glucan was depolymerized through cavitation effects. The high energy of cavitation resulted in a greater amount of β-glucan debris and smaller depolymerized molecules than was the case for the stretching effect exerted by mechanical shearing. This is the physico-chemical action of the high pressure homogenizer on the polymers [15]. The depolymerization of β-glucan using a high pressure homogenizer is a green chemical process for potential medical, pharmaceutical and food industry applications.

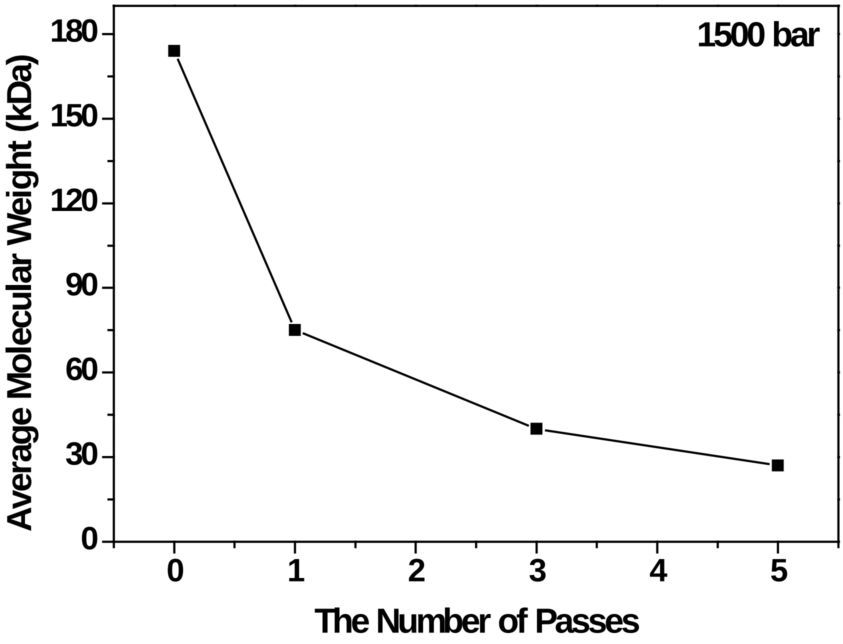

Figure 3. Effect of homogenization pressure and number of passes on the molecular weight of β-glucan.

3.3. FT-IR Spectrum of β-glucan

To confirm the breakdown of the glycosidic bonds, the β-glucan was subjected to FT-IR analysis before and after high pressure homogenization. Figure 4 shows the FT-IR spectra of the original β-glucan and depolymerized β-glucan by high pressure homogenization with 5 passes at 1500 bar. In the infrared region of the spectra corresponding to functional groups, there are major peaks at 3415, 2929 and 1748 cm^{-1}, which correspond to the stretching-absorption bands of O-H, C–H and C=O, respectively. The bands in the range 1158–1026 cm^{-1} are assigned to the characteristics of glycosidic linkage in the β-glucan. The peak at 890 cm^{-1} shows the β-D-glucan spectrum from the α-D-glucan spectrum [8,16,17]. The FT-IR spectra suggest that there were no significant differences between the chemical structure of the depolymerized and the original β-glucan.

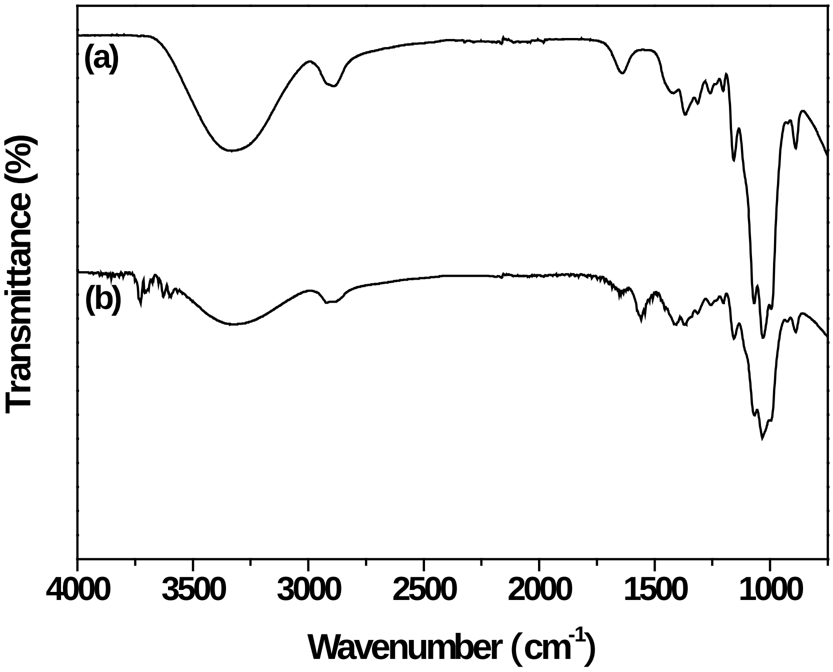

Figure 4. FT-IR spectra of (a) original β-glucan and (b) depolymerized β-glucan with 5 passes at 1500 bar.

3.4. UV-Vis spectrum of β-glucan solution

Figure 5 shows the UV-Vis spectra of the original β-glucan and the depolymerized β-glucan by high pressure homogenization with 1 to 5 passes at 1500 bar. The absorption band of the β-glucan solution was observed at 260 nm, which was ascribed to carbonyl groups [18]. The relative absorption intensity of the peaks at 1500 bar increased as the number of passes increased. This result indicates that the carbonyl groups might be formed during high pressure homogenization by breakage of the glycosidic bonds [19]. This result is consistent with other studies on polysaccharides.

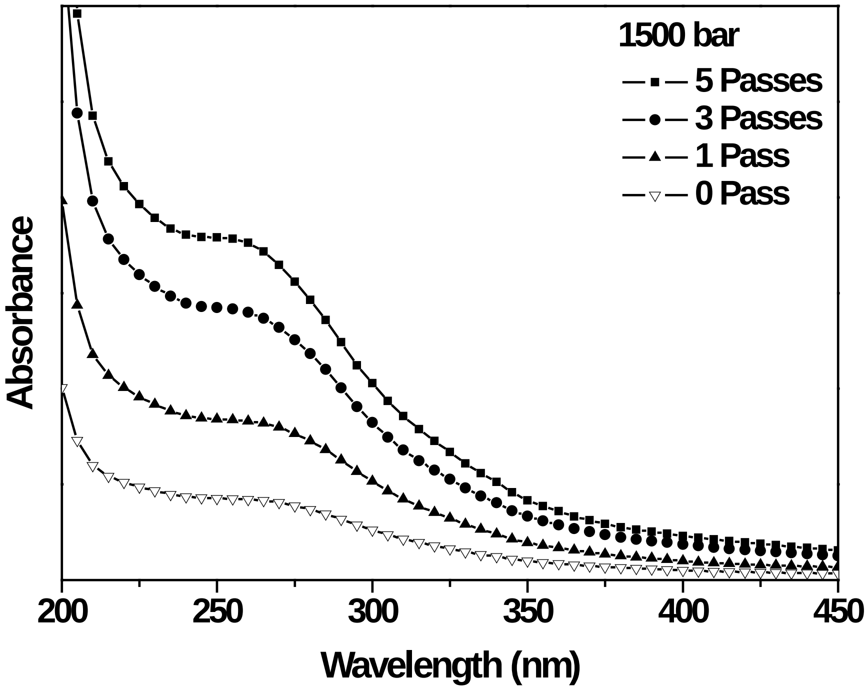

Figure 5. UV spectrum of original β-glucan and depolymerized β-glucan.

3.5. XRD Patterns of β-glucan

The XRD patterns of the original β-glucan and the depolymerized β-glucan are shown in Figure 6. The XRD patterns of the original β-glucan show diffraction peaks at 2 Θ = 5.9 °, 10.1 ° and 19.6 ° which match well with the literature values of polysaccharide polymers [20,21]. The intensity characteristics of the peaks under high pressure homogenization tend to decrease as the number of passes increase. A decrease in crystallinity occurs due to the destruction of the crystal structure of the β-glucan as a result of cleavage of the glycosyl bonds [6,22]. This could be attributed to the high energy shear, cavitation and impact effects during high pressure homogenization. This result appears to be in good agreement with the data on the viscosity and molecular weight of the β-glucan.

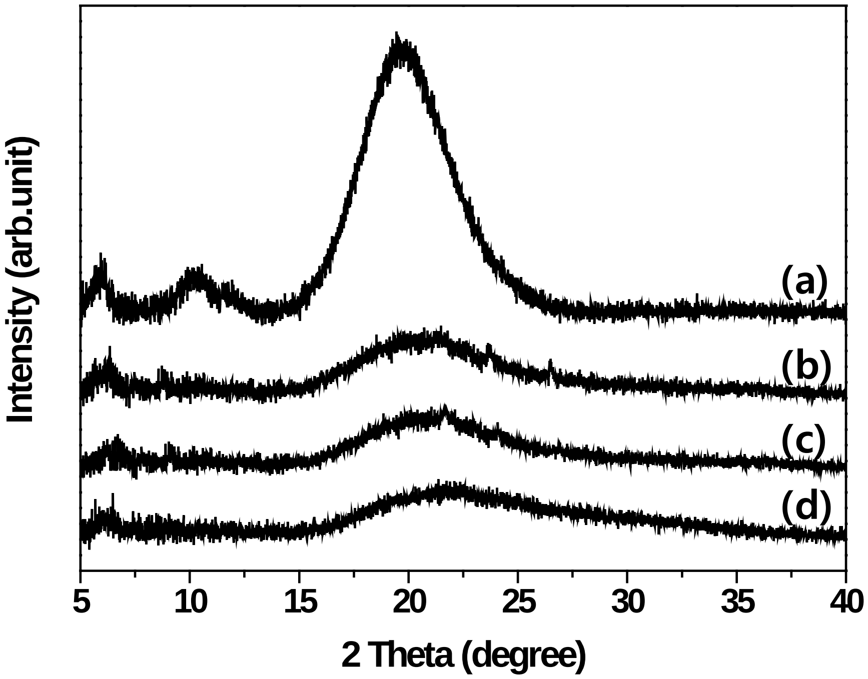

Figure 6. XRD patterns of the original β-glucan and depolymerized β-glucan.
(**a**) original β-glucan; (**b**) 1 pass; (**c**) 3 passes; (**d**) 5 passes.

4. Conclusions

The high molecular weight β-glucan was efficiently depolymerized by the use of a high pressure homogenizer. In the high pressure homogenization process, an increase in the pressure and the number of passes causes the viscosity and molecular weight to decrease without any chemical agent (acid/alkali) and without causing any notable changes in the functional-group status. In addition, the average molecular weight of the β-glucan can be efficiently controlled by the use of the pressure and the number of passes. The results of XRD and UV-Vis spectroscopy confirmed the claim that the reduction in the molecular weight of the resulting β-glucan led to the transformation of the crystal structure. The depolymerization of the β-glucan using the high pressure homogenizer is a green chemical process with medical, pharmaceutical, food and cosmetic industry applications.

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