International Journal of Biological Macromolecules 49 (2011) 369–377

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Rheological and microstructural investigation of oat β-glucan isolates varying in molecular weight

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ARTICLE INFO

Article history:
Received 25 October 2010
Received in revised form 5 May 2011
Accepted 18 May 2011
Available online 26 May 2011

Keywords:
Oat β-glucan
Molecular weight
Rheological properties
Microstructure
Atomic force microscopy

ABSTRACT

The rheological properties and microstructure of aqueous oat β-glucan solutions varying in molecular weight were investigated. The structural features and molecular weights (MW) were characterized by ^{13}C NMR spectroscopy and high performance size-exclusion chromatography (HPSEC), respectively. The microstructure of the β-glucans dispersions was also examined by atomic force microscopy (AFM). The samples with β-glucan content between 78 and 86% on a dry weight basis had MW, intrinsic viscosity ($[\eta]$) and critical concentration (c^*) in the range of 142–2800 × 10^3 g/mol, 1.7–7.2 dl/g and 0.25–1.10 g/dl, respectively. The flow and viscoelastic behaviour was highly dependent on MW and on the concentration of the β-glucans dispersions. Pseudoplastic behaviour was exhibited at high concentrations and Newtonian behaviour was evident at low concentrations. At the same concentration, the viscosity was higher for higher MW samples. The Cox–Merz rule was applicable for the lower molecular weight samples at higher concentrations whereas the high molecular weight sample deviated at concentrations greater than 1.0%, w/v. The mechanical spectra with variation of both MW and concentration were typical of entangled biopolymer solutions. AFM images revealed the formation of clusters or aggregates linked via individual polymer chains scattered heterogeneously throughout the system. The aggregate size increased with the molecular weight of the samples investigated and has been linked to the rheological behaviour of the samples.

1. Introduction

Oat (*Avena sativa* L.) and its products exhibit significant positive health effects including cholesterol lowering, modulation of glucose and insulin responses, weight management and improved gastrointestinal function. This is attributed to β-glucans, which have been accepted as a functional, bioactive ingredient [1–6].

β-Glucans in cereals are the predominant component of the endosperm cell walls of the grains. They are located throughout the starchy endosperm and are concentrated in the sub-aleurone layer. Their content ranges from 1% in wheat grains to 3–9% in oats, and 5–11% in barley [7,8]. β-Glucans are linear homopolysaccharides of consecutively linked (1→4)-β-D-glucosyl residues that are separated by single (1→3) linkages. The polysaccharide is neutral and made up of approximately 70% β-(1→4) and 30% β-(1→3)-linkages [9] and the molecular weight (MW) values for β-glucans range between 20 and 3100 × 10^3 g/mol depending on the botanical source, extraction/isolation protocols, and the analytical methodology used in the determination of these values [1,10–12].

Solubility in water and the capacity to form highly viscous solutions is a fundamental characteristic of oat β-glucans due to their high molecular weight, conformation, and self-association characteristics [2,12]. Fundamental studies on the rheological properties of β-glucans have been performed previously and the functionality of β-glucans was found to be highly dependent on polysaccharide structure, molecular weight and concentration [7,12–16]. However, limited information exists on the link between the microstructure and the rheological properties of this polysaccharide. Application of atomic force microscopy (AFM) has enabled visualization of individual polysaccharide molecules and their interactions in solution [17–27]. Imaging biopolymers by AFM to characterize important factors such as molecular weight, morphology and the nature of self-association can provide useful information to explain functionality.

The objective of this investigation, therefore, is to find a relationship between rheological and microstructural properties of oat

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doi:10.1016/j.ijbiomac.2011.05.014

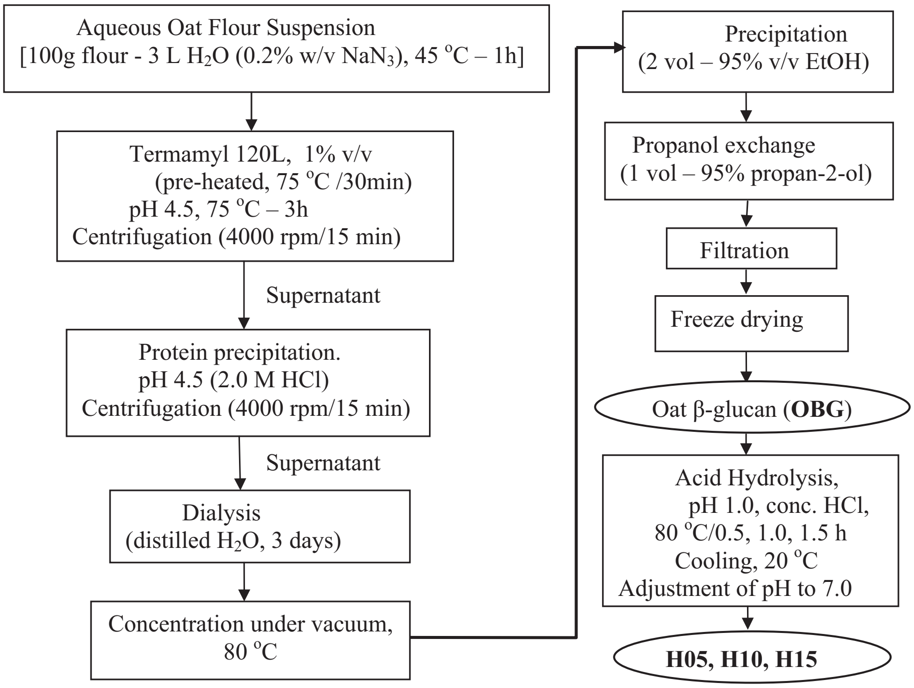

Fig. 1. Extraction and purification of β-glucan from oat flour.

β-glucans as a function of molecular weight using rheometry and AFM.

2. Materials and methods

2.1. Materials and chemicals

The Megazyme mixed-linkage beta-glucan assay and Quick Start^{TM} Bradford protein assay kits were purchased from Megazyme International Ltd (Bray, Ireland) and Bio-Rad Laboratories Inc. (Hercules, California, USA), respectively. Proteinase K-Agarose (from *Tritirachium album*), sodium azide (NaN_3), dialysis membrane tubing (MWCO 12000) and closures were purchased from Sigma–Aldrich (Poole, Dorset, UK) whereas ethanol and isopropanol were obtained from Fisher Scientific (Loughborough, UK). Termamyl 120L Type L (heat-stable alpha-amylase) was purchased from Univar (Bradford, UK). Oat flour (OatWell$^{®}$ 28%, an oat bran with 28% β-glucan content) was obtained from CreaNutrition (Swedish Oat fibre, Sweden). Distilled water was used throughout the experiments. All chemicals used were analytical grade reagents.

2.2. Extraction and purification of β-glucans

Extraction of oat β-glucans from oat flour was performed by adapting previously published isolation protocols with slight modifications [7,12,14]. Fig. 1 presents a schematic diagram of the extraction procedures employed for the isolation and purification of β-glucans. The extraction resulted in the initial β-glucan isolate that was denoted as OBG.

2.3. Acid hydrolysis

Three more samples (H05, H10, H15) were obtained from the initial isolate (OBG) by controlled acid hydrolysis. The β-glucan sample was dispersed in double-distilled water (1.5%, w/v) at 80 °C under continuous stirring in a sealed vial. When the polysaccharide was fully dispersed, the temperature was lowered to 70 °C and concentrated HCl added to bring the concentration to 0.1 M. The polysaccharide was hydrolysed for 0.5, 1, and 1.5 h and, immediately after the end of hydrolysis, the solutions were cooled in running water to room temperature, and the pH was adjusted to 7.0 with 5 M NaOH. The hydrolysates were precipitated with three volumes of 95% (v/v) ethanol and left standing for 1 h at 4 °C. The precipitate was collected by filtration, washed with isopropyl alcohol, freeze dried and ground to a powder of 600 μm mesh size.

2.4. β-Glucan and protein content determination

The β-glucan content of the isolates produced was determined by the McCleary method using the Megazyme mixed-linkage beta-glucan assay kit [28] and calculated using the Megazyme Mega-Calc^{TM} Software. The protein content was determined according to the Bradford protein assay [29]. A standard calibration curve was generated with bovine serum albumin (BSA) standards (125–1000 μg/ml) and used to estimate the protein content of samples. Absorbance readings were taken at 595 nm using a spectrophotometer (Shimazu UV-VIS 160A).

2.5. ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy

The ^{13}C NMR spectroscopy was performed with a Bruker AV 500 Spectrometer at 125.76 MHz using a 5 mm PABBO probe. The samples were dispersed (2%, w/v) in pure deuterated methylsulphoxide (d6-DMSO) by heating and continuous stirring at 90 °C for 3 h. The proton-decoupled spectra were recorded at 70 °C overnight by applying 12,800 pulses with a delay time of 2 s and a radio frequency angle of 30°. Chemical shifts were expressed in parts per million (ppm) relative to d6-DMSO at 39.5 ppm and reported relative to tetramethylsilane (Me_4Si).

2.6. Molecular weight determination

Purified gums were solubilized (~1 mg/ml) in deionised water for 3 h at 90 °C. Solutions were diluted with deionised water, filtered through a 0.45 μm filter, and the peak molecular weight (M_p) was measured by high-performance size-exclusion chromatogra-