Food Hydrocolloids 26 (2012) 175–180

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Solution behavior of barley β-glucan as studied with asymmetrical flow field-flow fractionation

Matilda Ulmius [a,*], Gunilla Önning [a], Lars Nilsson [b]

[a] Biomedical Nutrition, Pure and Applied Biochemistry, Department of Chemistry, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden
[b] Food Technology, Department of Food Technology, Engineering and Nutrition, Lund University, P.O. Box 124, SE-221 00, Lund, Sweden

ARTICLE INFO

Article history:
Received 2 February 2011
Accepted 9 May 2011

Keywords:
Cereal β-glucan
Aggregation
Molar mass
Field-flow fractionation
Multi-angle light scattering

ABSTRACT

Physicochemical properties of cereal β-glucans, associated with beneficial health effects, are related to their solution behavior and possibly to their propensity to form aggregates. Such properties are often analyzed with methods that may influence the aggregates *per se*. In this paper, the effect of processing on solution behavior of pure barley β-glucan was studied using asymmetrical flow field-flow fractionation (AsFlFFF), a method which is capable of analyzing the present aggregates. Molar mass distributions were determined by in-line multi-angle light scattering and refractive index detectors. Unprocessed samples had a main fraction of aggregates with a weight-average molar mass of 2.8×10^6 g/mol. Microwave heating to 100 °C reduced the largest aggregates, while heating to 121 °C prominently decreased the molar mass. Frozen storage for 1 week did not influence the aggregation, but repeated freeze–thaw cycles changed the structure of aggregates in a way that suggests cryogelation. The influence of processing conditions on solution behavior might explain why differently processed food products containing β-glucan have given different health effects. Experiments with the aim to eliminate aggregates demonstrated that filtration (0.45 μm) prior to analysis resulted in disruption of the largest aggregates, indicating that these aggregates will not be detected when filtration is used. Dissolution in NaOH solution, one of few solvents reported to eliminate aggregates, resulted in retained molar mass. Using AsFlFFF to study the solution behavior of β-glucans is a gentle method to analyze subtle changes of physicochemical properties.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Cereal β-glucan is a polysaccharide, which consists of linear chains of β-D-glucopyranosyl units linked via (1→3) and (1→4) linkages. β-Glucan is acknowledged as a functional and bioactive food ingredient (Lazaridou, Biliaderis, & Izydorczyk, 2007), and its physical and physiological properties have gained much interest due to its hypocholesterolemic and hypoglycemic effects (Biörklund, van Rees, Mensink, & Önning, 2005; Naumann et al., 2006). The functional properties are related to its solution behavior, i.e. the ability to form viscous solutions in the gut. β-Glucan molecules have the ability to self-associate and form aggregates, which may contribute to increased viscosity (Cui & Wang, 2009; Wood, 2004).

β-Glucan aggregates have previously been detected using batch light scattering (Grimm, Krüger, & Burchard, 1995) or been visually analyzed by confocal scanning laser microscopy (Wu et al., 2006). Such studies have reported both a fast and dynamic formation (Li, Cui, Wang, & Yada, 2011) and a slow formation, growing over several weeks (Kivelä, Gates, & Sontag-Strohm, 2009a). Many studies have tried to eliminate the aggregates by physical and/or chemical means, demonstrating that microwave heating to 100 °C for 10 min, 121 °C (corresponds to autoclave temperature) for 3 min, dissolution in 0.5 M NaOH or an ammoniacal $Cu(OH)_2$/CuCl-solution can eliminate the aggregates (Johansson, Karesoja, Ekholm, Virkki, & Tenhu, 2008; Li, Cui, & Wang, 2006; Wang, Wood, & Cui, 2002). β-Glucan aggregates are also reported to be disrupted by shear forces. This might explain the difficulty encountered when analyzing aggregates using high-performance size-exclusion chromatography (HPSEC), since high shear forces are present in the column (Gómez, Navarro, Manzanares, Horta, & Carbonell, 1997; Li, Wang, Cui, Huang, & Kakuda, 2006).

Furthermore, β-glucans are known to be degraded during food processing, which can influence the above mentioned physiological effects. Most studies have measured the effect of cooking, processing and storage according to the molar mass as determined by HPSEC (Beer, Wood, Weisz, & Fillion, 1997; Wood, 2007), although

* Corresponding author. Tel.: +46 46 222 96 07; fax: +46 46 222 46 11.
E-mail address: matilda.ulmius@tbiokem.lth.se (M. Ulmius).

0268-005X/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodhyd.2011.05.004

the examined molar mass may have been affected by the analytical method *per se*.

Asymmetrical flow field-flow fractionation (AsFlFFF) is a gentle separation method due to its absence of a stationary phase in the separation channel (Giddings, 1993). The method allows for fractionation of samples with a wide range of particle sizes (about 2 nm to above 10 μm), which makes it suitable for analyzing β-glucan molecules and aggregates, as previously shown in the characterization of bacterial β-glucans (Lambo-Fodje et al., 2007). The separation in AsFlFFF is obtained by a transport flow and a perpendicular field (cross-flow), which transports the sample components towards an accumulation wall (Wahlund & Giddings, 1987; Wittgren & Wahlund, 1997). Small particles, having stronger diffusion than large ones, will have longer average distance from the accumulation wall and will hence elute earlier from the fractionation channel. Molar mass distribution can be determined by connecting the AsFlFFF in-line to a multi-angle light scattering (MALS) detector and a refractive index (RI) detector. The aim of this study is to use AsFlFFF to examine changes in the solution behavior as a result of processing of pure barley β-glucan. This includes i) a confirmation that the method is not influencing the solution state, i.e. aggregate formation, ii) molar mass analyses after processing similar to food processing and a study of the effect of storage, and iii) molar mass analyses after processing that may eliminate the aggregates.

2. Materials and methods

2.1. Preparation of solutions

Barley β-glucan standard (weight-average molar mass (M_w) 359 000 g/mol, as determined by HPSEC in 0.05 M NaOH, boiling 10 min during dissolution prior to analysis), >95% pure, was purchased from Megazyme International Ltd, Bray, Ireland. Sample solutions with β-glucan, 0.25% (w/v), were prepared with the carrier liquids for the AsFlFFF as described subsequently; 10 mM $NaNO_3$, 0.05 M NaOH or 0.5 M NaOH. Preparation of β-glucan samples in $NaNO_3$ required magnetic stirring at 70 °C for 30 min while dissolution in NaOH was performed through stirring at room temperature for 30 min.

When evaluating any influence of AsFlFFF on β-glucan aggregate formation, dextran (from Leuconostoc mesenteroides; M_w 2.0×10^6 g/mol, Sigma–Aldrich Co., St Louis, MO), 0.25% (w/v), was tested as a comparison, dissolved in 10 mM $NaNO_3$ by stirring at room temperature for 30 min.

2.2. Instrumental set-up

The AsFlFFF instrument (Eclipse 3+ Separation System, Wyatt Technology Europe, Dernbach, Germany) was connected to a MALS detector (Dawn Heleos II, Wyatt Technology) operating at a wavelength of 658 nm and an RI detector (Optilab DSP, Wyatt Technology) operating at 632.8 nm. A pump (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) coupled to an in-line vacuum degasser delivered the carrier flow. To ensure a particle-free carrier flow into the channel, a filter (100 nm pore size, polyvinylidene fluoride filter, Millipore Corp., Bedford, MA) was placed after the pump. An auto sampler (Agilent 1100, Agilent Technologies) handled the sample injection. The AsFlFFF channel (Wyatt Technology) had a length of 26.5 cm and a nominal thickness of 190 μm, while the actual thickness was determined to be 162 μm by calibration against ferritin, as described by Litzen (1993). The membrane forming the accumulation wall was made of polyethersulphone with a cut-off of 10 kDa (Microdyn-Nadir GmbH, Wiesbaden, Germany).

Three different carrier liquids were used; i) 10 mM $NaNO_3$ (Analytical grade, Merck, Darmstedt, Germany) containing 0.02% (w/v) NaN_3 (Analytical grade, Merck) to prevent bacterial growth and was filtered through a 0.2 μm pore-size cellulose acetate filter (Whatman International, Maidstone, UK) prior to use, ii) 0.05 M NaOH (Reagent grade, Sigma–Aldrich) as used for molar mass determination by HPSEC (Megazyme, 2010) and iii) 0.5 M NaOH in accordance with Li, Cui, et al. (2006) and Li, Wang, et al. (2006) who demonstrated that no β-glucan aggregates were detectable by batch light scattering in this medium.

2.3. AsFlFFF procedure

The AsFlFFF analysis contains three phases: injection/focusing (relaxation), elution and rinsing. In the injection/focusing phase, 50 μl (0.125 mg β-glucan) of sample was injected at a rate of 0.2 ml/min during 1 min. Before separation, the sample was focused at a point where the two streams of flow meet to let it be arranged at different distances from the membrane. Various focusing times were tested when evaluating influence of AsFlFFF conditions on aggregate formation (see below), and a focusing time of 4 min was chosen for all other analyses. In the elution phase, the inlet flow was divided into an axial flow through the channel and a perpendicular flow crossing the membrane. The elution was started at an initial cross flow of 0.6 ml/min which was decreased linearly with 0.09 ml/min, i.e. the cross flow was zero after 7 min and then kept constant at zero for 10 min. After elution, the channel was rinsed without cross flow for 10 min before the next analysis. All injections were carried out at room temperature and the detector flow was constant at 1.0 ml/min throughout the separation.

2.3.1. Effect of focusing times on aggregation

A long focusing time may increase the risk of sample-membrane or sample–sample interaction (Pazourek & Chmelík, 1993), i.e. the focusing might induce aggregation. To evaluate if the focusing phase had any influence on β-glucan aggregate formation, β-glucan or dextran was dissolved in $NaNO_3$, and injected using different focusing times (including 1 min focusing/injection) of 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6 and 11 min.

2.3.2. Effects of food processing, filtration or storage

AsFlFFF analysis was performed on 4 unprocessed pure β-glucan samples, dissolved in $NaNO_3$. One of these was tested for focusing times (above), while another was divided into aliquots in order to test the change in solution behavior of β-glucan after various processing methods. Several samples (not aliquots) were tested after either of the processing steps with similar results (not shown). The effect of heating was followed using a microwave heater (Mars 5, CEM Corporation, Matthews, NC) at 100 or 121 °C for 4 and 10 min, sealed in a high-pressure vessel (HP-500, CEM Corporation) in accordance with Wang et al. (2002). Freezing and freeze–thaw cycling processes were tested, either by storing samples (1 ml) at −20 °C for 1 week, or removing them from the freezer after 1 day to stand at room temperature for approximately 2 h until completely thawed; and then keeping them in the freezer for at least 5 h. Freeze–thaw cycles were repeated 5 or 10 times before analysis. Furthermore, the effect of filtration through a 0.45 μm filter (Acrodisc; Pall Corporation, Ann Arbor, MI) prior to injection was tested. The effect of storage at room temperature was tested on the unprocessed sample (i.e. dissolved in $NaNO_3$, 70 °C for 30 min), heated to 121 °C, and the filtered sample. These were analyzed once a week for three weeks.

2.4. Calculations

Analysis of the light scattering data was performed using the Astra software (version 5.3.4.14, Wyatt Technology). Baseline corrections for the RI signal were performed by blank baseline

Download English Version:

https://daneshyari.com/en/article/604994

Download Persian Version:

https://daneshyari.com/article/604994

Daneshyari.com