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Enhanced separation and characterization of gluten polymers by asymmetrical flow field-flow fractionation coupled with multiple detectors

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ABSTRACT

Asymmetrical flow field-flow fractionation (AsFlFFF) coupled with refractive index (RI) and multi-angle light scattering (MALS) detectors was used for macromolecular characterization of four different industrial wheat protein preparations (native, enzymatically hydrolyzed, physically separated, and denatured). The fractionation conditions were optimized separately for each protein sample and molar masses were determined from RI and MALS signals. Decaying cross-flow gradient seemed to produce best results for most of the gluten samples in terms of resolution and sample recovery. Sonication of the samples enabled the solubilization of the high-molar mass components with molar mass ranging from 8×10^6 to 3.5×10^8 g/mol. In case of lower-molar mass glutenins (α-gliadins, ω-gliadins, and high molecular weight glutenin subunits), AsFlFFF results were also compared with the results obtained with capillary gel electrophoresis.

1. Introduction

Gluten is a protein fraction of wheat responsible for the unique dough-forming characteristics of wheat flour. About 80% of total wheat protein belongs to the gluten proteins. Gluten proteins affect many of the quality attributes of bread such as volume, crumb structure and crispness and extra gluten is often added to weak wheat flours to improve their baking quality. Gluten proteins are divided into gliadins and glutenins which are responsible for extensibility and elasticity of wheat dough, respectively. Gluten proteins are among the largest proteins in nature. Especially glutenins are able to form polymers of several million in molar mass (Wahlund et al., 1996).

The molecular sizes of the largest gluten polymers are difficult to analyze, due to their low solubility and high hydrophobicity. Native gluten polymers, especially high-molar mass fraction, cannot be easily solubilized which complicates their solution characterization. Detergents, such as sodium dodecyl sulfate (SDS), has been used to increase the solubility (Wahlund et al., 1996), but when using chaotropic detergents, the protein structure changes due to micelle formation. Up to 95% of flour protein can be extracted by SDS (Bottomley et al., 1982); however, the ability of SDS to form micelles with proteins disturbs the analysis of protein size and the results obtained with a presence of SDS were not reproducible (Stevenson and Preston, 1996). Acetic acid has shown to be quite efficient in solubilizing gluten proteins. Up to 86% of gluten proteins can be solubilized in 0.01 M acetic acid (Popineau, 1985). Also 1-propanol has turned out to be very efficient in solubilizing both gluten components; monomeric gliadins and polymeric glutenins (Bean et al., 1998). Sonication is one of the methods that can be used to improve the solubility of large molecules. Sonication, however, may cause breakdown of largest polymers and cause underestimation of true polymer sizes.

Size-exclusion chromatography (SEC) and field-flow fractionation (FFF) are the methods commonly used for the separation and macromolecular characterization of glutenin polymers (Bean and Lookhart, 2001; Mendichi et al., 2008; Stevenson et al., 1999; Stevenson and Preston, 1996; Wahlund et al., 1996). Especially FFF, in which separation takes places in an open channel instead of packed columns used in SEC, has been proposed to be applicable for large-size gluten polymers. Both symmetrical and asymmetrical FFF (AsFlFFF)

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with ultra violet detection (UV) has been used for separation and characterization of wheat proteins (Stevenson et al., 1999; Stevenson and Preston, 1996; Wahlund et al., 1996). In these studies, the hydrodynamic diameters have been calculated from the observed retention times based on the FFF theory or by using well-known proteins as size standards. When the molar mass and size (radius of gyration, R_g) are determined by static light scattering, the results are not so susceptible to the deviations from the FFF retention theory and the cross-flow gradient can be freely optimized for each sample. Overloading of the FFF channel, for instance, can cause bias to the hydrodynamic diameters if the calculations are based on the relationship between size and observed retention time. Only a few reports were found describing the characterization of wheat proteins by FFF coupled to refractive index (RI) and multi-angle light scattering (MALS) detectors (Arfvidsson et al., 2004; Stevenson et al., 2003).

In addition to finding optimal solubilization conditions for wheat proteins and carrier liquid composition for FFF, the minimization of possible protein-membrane interactions may be challenging in FFF. Ideally, no interactions between membrane and analyte should exist but this is often difficult to achieve. Kassalainen and Williams (2012) studied protein-membrane interactions using bovine serum albumin (BSA) and γ-globulin and different membrane materials (regenerated cellulose and polyethersulfone) with varying elution conditions. For both of these model proteins the recovery, at any conditions, never reached 100%.

In this study, we optimized the AsFlFFF fractionation conditions for different wheat gluten samples (native, denatured, physically separated, and enzymatically hydrolyzed) with varying mass/size distributions. The main focus was in finding the best possible fractionation conditions with minimum protein-membrane interactions and maximum analysis recovery values. Our aim was to characterize the molar mass distribution of different gluten preparations including the polymeric fractions with very high molar masses.

2. Experimental

2.1. Sample material

The gluten samples used in this study included two native glutens (Vital Wheat Gluten, Kröner-Stärke, Ibbenbüren, Germany and Vital Gluten, Raisio, Finland), two enzymatically hydrolyzed and one physically separated gluten samples marketed as dough modifiers (Meripro 410, Meripro 500 and Meripro 810, Tereos Syral, France), and one denatured gluten (Glustar 312, Kröner-Stärke, Ibbenbüren, Germany). Protein contents analyzed by the Dumas combustion method (Vario MAX CN, Germany) using N x 5.7 (AACC Method 46-30) ranged from 69% to 78% between studied gluten samples.

2.2. Sample preparation

The samples were dissolved in 0.05 M acetic acid (pH 3.0) with a concentration of 1 mg/ml, incubated overnight at room temperature and centrifuged at 3220*g* for 20 min. Sonication was used to enhance the dissolution of the polymeric proteins from native gluten samples as described by Ueno et al. (2002). Sonicated samples were prepared by mixing the precipitant of the previous extraction with 1 ml of 0.05 M acetic acid, and sonicating the suspension with 45 W for 60 s. Increasing the sonication power to 75 W resulted in a slight decrease in the recovery of proteins with higher molar mass. The soluble protein concentrations of the extracts were analyzed by the Lowry method using Bio-Rad DC protein assay (Bio-Rad Laboratories Inc., USA). A gliadin standard prepared by the Prolamin Working Group was used as the standard. The sample solutions were filtered using 0.45 μm syringe filters prior to AsFlFFF analysis.

2.3. Automated capillary gel electrophoresis

The compositions of acetic acid soluble gluten proteins were investigated by the automated capillary gel electrophoresis system (Experion, Bio-Rad Laboratories Inc., USA). The method is based on a similar separation of proteins by their sizes as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The sample proteins are solubilized with a buffer containing SDS and the separation is achieved by the electric current moving the charged SDS-protein micelles through capillaries filled with gel material. Experion Pro260 Analysis Kit (700-7101, BioRad Laboratories, USA) was used in the separation of gluten proteins following the instructions of the manufacturer. Analysis range was between 3500 and 260 000 g/mol. All the samples were analyzed under reducing conditions.

2.4. AsFlFFF

In AsFlFFF, the separation takes place in an open channel. The channel consists of porous bottom frit covered with membrane, a spacer which defines the channel thickness and top plate. After the sample components are transported to the channel, they start to form equilibrium distributions in different flow velocity streamlines of the parabolic flow existing in the channel. The flow perpendicular to the parabolic flow, so called cross-flow, is used to enhance the separation of sample components with different hydrodynamic volume. In principle, the separation in AsFlFFF is based on the diffusion coefficients (D) of the eluted molecular species and by determination of D values from the retention times, the hydrodynamic diameters can be further calculated using the Stokes–Einstein relationship. Generally, the constant cross-flow is used in the analyses aiming to estimate the hydrodynamic diameter from the retention time, as the determination of D from retention times is more straightforward in comparison with the analyses where the cross-flow gradient is used (Nilsson et al., 2006). In our analyses, we tested different cross-flow gradients (constant cross-flows of 2 and 3 ml/min, and linearly decaying cross-flows of 1, 2, and 3 ml/min) and employed MALS/RI detection for mass and size calculations.

AsFlFFF experiments were carried out using an AF2000 MT instrument (including software, Postnova Analytics, Landsberg/Lech, Germany) equipped with UV (λ = 210 nm, Shimadzu SPD-20A, Kyoto, Japan), MALS (Brookhaven Instruments Corporation, Holtsville, NY, USA), and RI (PN 3150, Postnova Analytics) detectors (Pitkänen et al., 2011). The MALS detector contains 30 mW laser as the light source operating at λ_0 = 660 nm with seven scattering angles (35°, 50°, 75°, 90°, 105°, 130°, 145°). The eluent used was 0.05 M acetic acid. Regenerated cellulose (RC) membrane with a nominal cut-off value of 10 000 g/mol and a spacer with a thickness of 350 μm were used in the separation channel. The detector flow rate was 1 ml/min. All samples were also analyzed without cross-flow to estimate the total analysis recovery. The focusing times tested were 1, 2, and 3 min. The RI and MALS detectors were calibrated and normalized according to instructions from Postnova Analytics (2009) using the bovine serum albumin and polystyrene sodium sulfonate standards. The Zimm equation (first order) was used for fitting the light scattering data. The injection volume was 100 μl.

3. Results and discussion

3.1. Solubility of gluten

Solubility of gluten in 0.05 M acetic acid was generally very good according to Lowry analyses (Table 1) indicating that 74% and 71% of total protein of vital gluten samples and all of the proteins in