Characterization of Gluten Protein Using Asymmetrical Flow Field-Flow Fractionation Coupled with Multi Angle Light Scattering

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Application	Protein, Food
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Introduction

Gluten proteins have become increasingly important in recent years due to the rise in Celiac disease, which affects up to 5% of the world's population [1], and associated gluten-free diets. One of the challenges for the analysis of gluten is their relatively high molar mass and consequent large molecular size. The glutenin fraction of glutens can be as large as 10 million Daltons [2], making them difficult to separate and characterize by column-based chromatography techniques such as size exclusion chromatography. Often, gluten samples will be sonicated prior to chromatographic separation, and the resulting fragments will be analyzed. In this application note, we present data on separation of native gluten using Asymmetrical Flow Field-Flow Fractionation (AF4) with no sample pre-treatment, which can provide a more direct measurement of their molecular weight and radius of gyration (R_g) distributions.
A schematic for the AF4 channel is shown in Figure 1. The combination of cross flow and channel flow causes size separation over the course of the analysis, with smaller particles eluting to the connected detectors before larger particles, including aggregates and agglomerates.

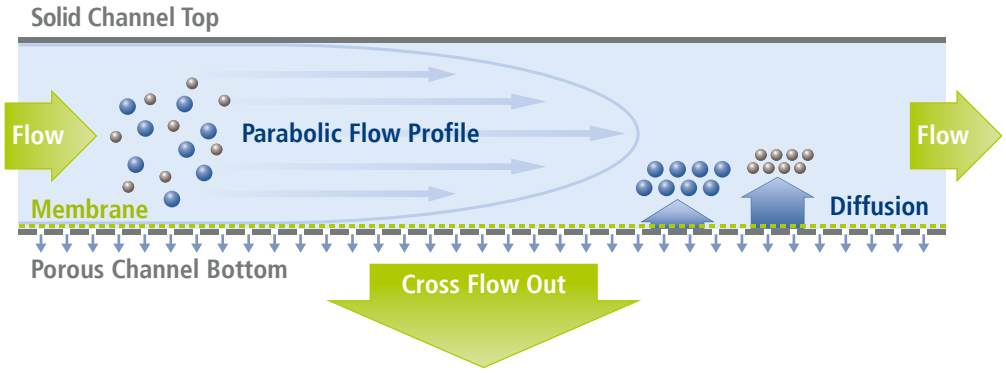

Figure 1: Schematic of the AF4 separation principle.

Experimental Details and Results

To separate glutens by size and characterize them, an AF4 system (Postnova AF2000) was used with two detectors monitoring the eluent. Firstly, a Postnova 21-angle multi angle light scattering detector (MALS, PN3621) for measuring the R_g of glutens and their aggregates and agglomerates; and secondly a refractive index detector (RI, PN3150) which is sensitive to protein concentration and, in combination with MALS, can provide molecular weight measurements. A carrier solution of 70 mM acetic acid was used.

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For the investigated gluten samples, we see molecular weights ranging from around 30 kDa to 100 MDa (Fig. 2). The three samples are distinct from each other with respect to molecular weight. The lower end of these values is consistent with the established literature on the monomeric forms, called gliadins [2]. The upper end of molecular weights is usually expected to be about 10 MDa, but we measure here larger values, around 100 MDa, which is most likely due to AF4's ability to separate these extremely large protein aggregates, where column chromatography would fail.

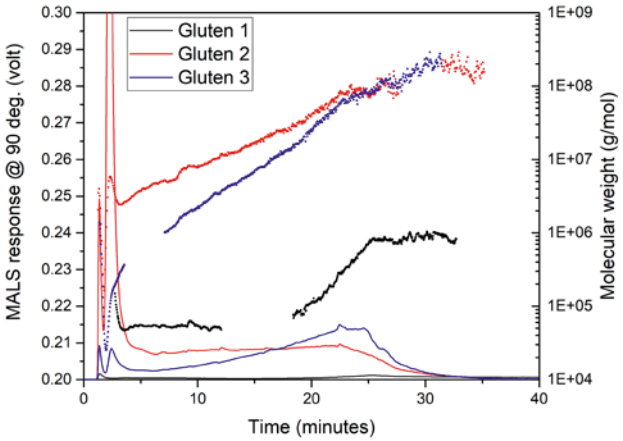

Figure 2. MALS-based fractograms (lines) overlaid with molecular weight values

In Figure 3, the MALS response is plotted with lines, and R_g calculated at each time point and plotted as dots. Just as was observed for molecular weight, we are able to differentiate the three samples by radius. For sample 3 especially, there is substantial polydispersity in the size distribution, ranging from about 50 nm to 500 nm in radius.

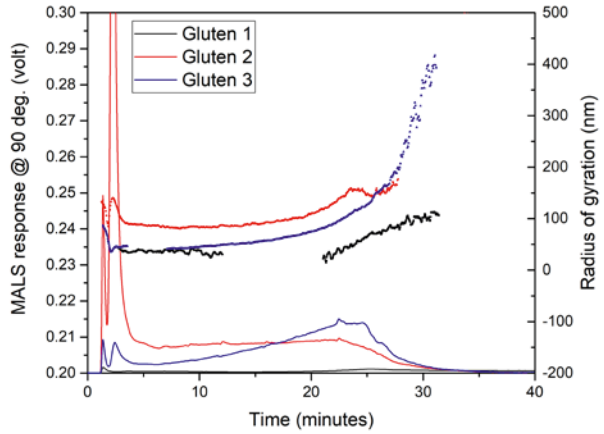

Figure 3. Radii of gyration (dots) plotted across representative fractograms (lines) for the gluten samples using AF4 -MALS.

Conclusion

From the data presented here, we see that AF4-MALS-RI can separate large gluten proteins and provide molecular weight and molecular size (R_g) values. For two of the samples analyzed, the upper end of the molecular weight was an order of magnitude higher than what is considered the typical upper end (~10 MDa). Most chromatography columns would filter out some or all gluten complexes that are this large resulting in incorrect determination of the gluten's overall size and molecular weight distribution.

References

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[2] H. Wieser, Food Microbiology, 2007, 24(2), 115-119.

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