

AN2302: Starch amylose and amylopectin molar mass and size distributions by AF4-MALS

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Introduction

Starch is used for a variety of industrial and nutritional purposes. Its functional properties are influenced by the ratio and molar masses of its macromolecular constituents, which vary with source, crop year, and climate. Starch contains large homopolymers of amylose (AMY) and amylopectin (AMP).

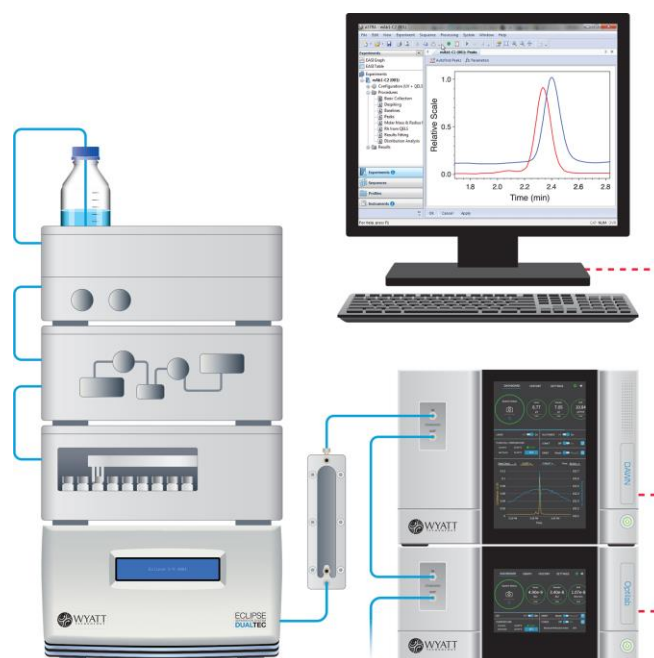
Linear AMY consists of long chains of (1 \Rightarrow 4)- α -D-glucose linkages, while the higher-molar-mass AMP is a branched structure containing a mixture of (1 \Rightarrow 4)- α - and (1 \Rightarrow 6)- α -D-glucose linked residues. With average radii in the hundreds of nm, and molecular weights ranging into the hundreds of millions, starch polymeric components cannot be separated by GPC. However, asymmetric-flow field-flow fractionation coupled to multi-angle light scattering (AF4-MALS) is suitable for separation and characterization of polymers and nanoparticles from 1 nm to 1000 nm and hence is applicable to starch analysis. AF4 performs non-shearing separation by hydrodynamic size, and MALS analyzes absolute molar mass and size regardless of conformation or retention properties.

Materials and Methods

The goal of this work was to apply AF4-MALS to separate AMY and AMP in order to calculate the AMY:AMP mass ratio, to determine the molar mass distributions, the average molecular weights of AMY and AMP, and the z-average root-mean-square radius $R_{g,z}$ and polydispersity M_w/M_n of the AMP component. We applied the technique to starches with AMY:AMP ratios covering a wide range.

An Eclipse® AF4 system was equipped with an 18-cm short channel, a 350 μ m spacer, and a 10 kDa cutoff, regenerated cellulose membrane. Detection was accom-

plished with DAWN® 18-angle MALS and Optilab® RI detectors. The channel flow was maintained at 1.0 mL/min and the cross-flow was varied linearly from 1.0 to 0.1 mL/min for 10 minutes, then abruptly switched to 0.0 mL/min. Data were collected and analyzed in ASTRA® software.



An FFF-MALS system includes HPLC components such as degasser, pump, autosampler and UV detector, plus a DAWN MALS detector and Optilab differential refractometer.

Results and Discussion

Figure 1 shows that AF4-RI fractograms separate into distinct peaks for amylose and amylopectin. The molar masses distributions determined for these samples ranged from 10 kDa to 1 GDa, though there is evidence for smaller starch components as well.

Table 1 summarizes the results of the analyses. Integration of the respective peak areas enabled calculation of the AMY:AMP ratios, in excellent agreement with the

nominal values. The values for M_w and $R_{g,z}$ fall within the generally accepted limits found in the literature.

Polymer [conformation](#) is assessed by comparing R_g with molar mass. Conformational plots for the AMP component, shown in Figure 2, verify its branched nature.

Species-specific differences in the overall density of the amylopectins lead to the vertical offsets of the Hi-maize and Hylon VII plots relative to the other three.

Table 1. AF4-MALS-RI results for 5 native starches of varying AMY:AMP ratio: measured ratio vs. nominal, molar mass, size and polydispersity results

Sample	AMY:AMP (nominal)	AMY:AMP (measured)	Amylose M_w (g/mol)	Amylopectin		
				M_w (g/mol)	$R_{g,z}$ (nm)	M_w/M_n
Hi-Maize	75:25	74:26	227,900	109,200,000	145	2.4
Hylon VII	75:25	77:23	204,000	94,340,000	158	1.9
Hylon V	55:45	56:44	367,000	251,100,000	245	2.5
Melojel	25:75	26:74	419,000	418,500,500	316	2.9
Amioca	1:99	1:99	---	321,000,000	261	2.1
Hi-Maize	75:25	74:26	227,900	109,200,000	145	2.4

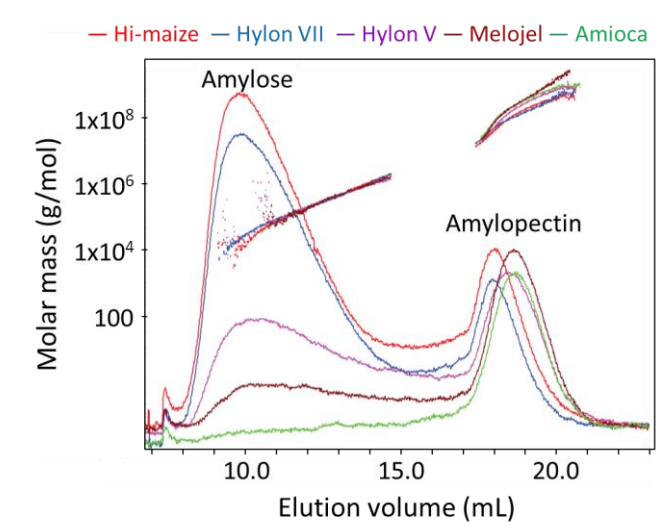


Figure 1. AF4-MALS-RI results for five native starches of varying AMY:AMP ratio; AF4-RI fractograms with molar mass distributions overlaid.

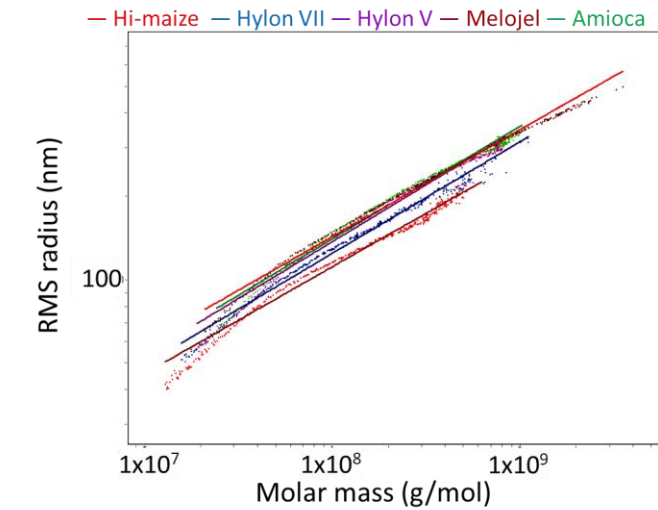


Figure 2. Conformation plot ($\log R_z$ vs. $\log M_w$) for the amylopectin component of 5 starches. Log-log slopes of 0.39-0.41 are indicative of branching.

Overcoming SEC limitations

Starch characterization by size-exclusion methods can be limited due to shear degradation and/or column adsorption of the higher mass fractions of AMP. The open-channel separation of AF4 eliminates these limitations and enables recovery of intact AMP.

AF4 is a versatile separation technique in that the separation power can be varied over the course of the method. Here, enhanced resolution of AMY and AMP was achieved by varying the cross-flow in gradient fashion.

Coupled with MALS and RI detection, the mass ratios of AMY:AMP were accurately determined and the molar masses of these macromolecules were estimated over five orders of magnitude.

Analytical SEC is not appropriate for amylose and amylopectin. SEC columns cannot cover the entire size range and may induce shear degradation. Due to branching, the usual linear calibration standards would not have been applicable. Only AF4-MALS fully addresses the multiple analytical challenges presented by starch



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