AFFF-MALS-RI for Determining the Mass and Size Distributions of Amylose and Amylopectins in Starch

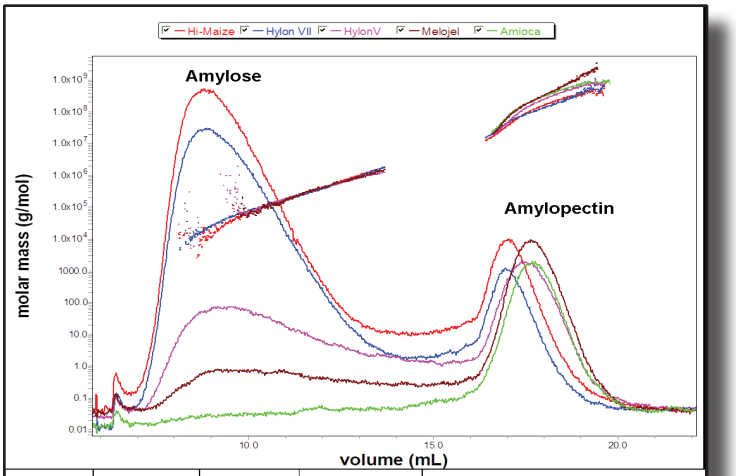
Starch is used for a variety of industrial and nutritional purposes. Its functional properties are influenced by the ratio and molar masses of its macromolecular constituents, which vary with source, crop year, and climate. Starch contains large homopolymers of amylose (AMY) and amylopectin (AMP).

Linear AMY consists of long chains of (1⇒4)-α-D-glucose linkages, while the higher molar mass AMP is a branched structure containing a mixture of (1⇒4)-α- and (1⇒6)-α-D-glucose linked residues. The goal of this work was to apply AF4-MALS-RI to separate AMY and AMP in order to calculate a mass ratio, to determine the molar mass distributions, the average molecular weights (M_w), and the mean-square radius (R_z) 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 a short (18 cm) channel, a 350 μm spacer, and a regenerated cellulose (10 kDa cutoff) membrane. Detection was accomplished with DAWN EOS 18-angle MALS and Optilab REX 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.

Integration of RI peak areas enabled calculation of the AMY:AMP ratios, in excellent agreement with the nominal values. The values for M_w and R_z fall within the generally accepted limits found in the literature. Conformational plots for the AMP component verify its branched nature.

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 obviates these limitations and enables recovery of intact AMP. Resolution of AMY and AMP was achieved by varying the cross-flow. Coupled with MALS and RI detection, the mass ratios of AMY:AMP were accurately determined and the molar masses of these macromolecules were estimated without the need for calibration standards.



Sample	AMY:AMP (nominal)	AMY:AMP (calc)	Amylose	Amylopectin		
			M_w (g mol^{-1})	M_w (g mol^{-1})	R_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	367,200	251,100,000	245	2.5
Melojel	25:75	26:74	419,700	418,500,000	316	2.9
Amioca	1:99	1:99	---	321,000,000	261	2.1

Figure 1. AF4-MALS-RI results for 5 native starches of varying AMY:AMP ratio: AF4-RI fractograms with molar mass distributions overlayed. (Cross-flow (V_x) = 1.0 to 0.1 mL min^{-1} in 10 min, then V_x = 0.0 mL min^{-1}).

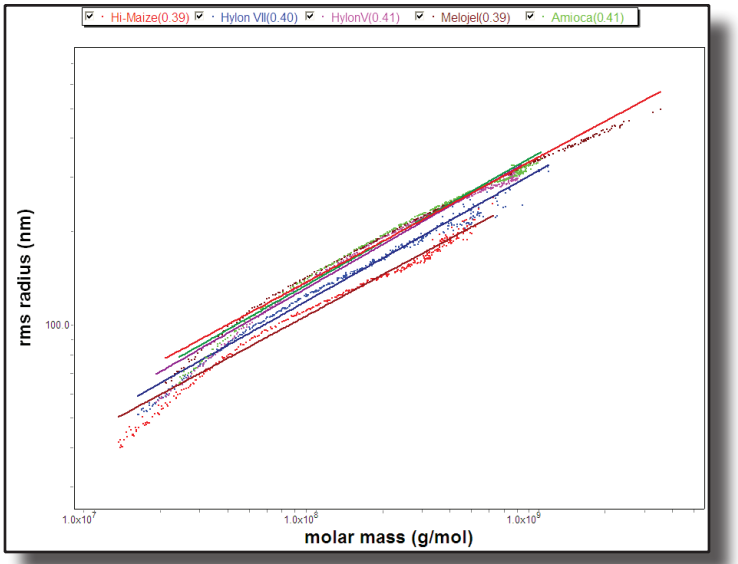


Figure 2. Conformation plot (log R_z vs. log M_w) for the amylopectin component of 5 starches (Slopes 0.39-0.41 indicative of branching).

This note graciously submitted by Rick White and Eija Chiaramonte, Global Analytical Sciences—Personal Health, The Procter & Gamble Company, Mason, OH.

Wyatt Technology CORPORATION
6300 Hollister Avenue • Santa Barbara, CA 93117
TEL (805) 681-9009 • FAX (805) 681-0123
E-mail: info@wyatt.com • URL http://www.wyatt.com