Fractionation of Industrial Starch Polysaccharides by Field-Flow Fractionation

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Objectives
- Determine best sample preparation techniques for the dissolution of starch in organic media
- Perform fractionation of amylose and amylopectin components of industrially important starch samples using thermal field-flow fractionation (ThFFF)
- Determine molecular weight (MW) for amylose and amylopectin

Background
Starches are important macromolecules with many industrial applications, particularly in the food and pharmaceutical industries. Their uses range from gelling agents to drug transport and even energy storage. Properties of starches vary based on the plant or bacterial source and are connected to the amylose and amylopectin content. Amylose is a linear polysaccharide with 1,4-glycoside bonds and has a molecular weight around 10^6 Daltons. Amylopectin is a highly branched polysaccharide with α-1,6-glycoside bonds along a α-1,4-glycoside bond chain backbone and has a molecular weight around 10^6 Daltons [1]. Characterization of starch has previously been performed using size-exclusion chromatography (SEC). The use of SEC for polysaccharides is limited due to low exclusion limits of the packed column and shear degradation of the ultrahigh molecular weight components. An alternative to SEC is field-flow fractionation (FFF). In 1994, Lou et al. reported the use of thermal FFF (ThFFF) to perform the separation of pullulan with varying molecular weight and of corn starch with varying amylose and amylopectin content [2]. ThFFF uses a temperature field applied perpendicular to the carrier liquid flow to promote the retention and fractionation of analytes based on the ratio of thermal diffusion coefficient D_t to normal diffusion coefficient D. The retention time of the analyte is therefore controlled by the temperature difference across the channel ΔT, the size and molecular weight of the analyte, and parameters that affect D_t.

Instrumentation
Thermal channel: Model T-101 (Postnova Analytics, Salt Lake City, UT)
Length: 32 cm  Breadth: 2 cm  Thickness: 0.0127 cm

Experimental
Starch samples with varying amylose content (0, 25, 50, 70%) were provided by National Starch and Chemical Company (Bridgewater, NJ). Carrier liquid was distilled dimethyl sulfide (DMSO), flow rate of 0.1 mL/min. Temperature programming was used: initial ΔT=80°C for 10 minutes then decayed to ΔT=10°C.

Sample Preparation
Method 1: starch cooked in distilled dimethyl sulfide (DMSO) in an oil bath at ~80°C for 3 days with constant stirring. Sample concentrations were 1.0 mg/mL.
Method 2: starch cooked in 80% aq. DMSO in a boiling water bath (94-96°C) for 1 hour with constant stirring. Sample was extracted when temperature was near room temperature. Sample concentration 2.0 mg/mL.

Results and Discussion for Cooking Method 1

Figure 1 – Fractogram of 100% amylose with molecular weight overlay. Sample prepared by Method 1.

Figure 2 – Fractogram of 50% amylose and 50% amylopectin with molecular weight overlay. Sample prepared by Method 1.

Results and Discussion for Cooking Method 2

Figure 3 – Fractogram of 50% amylose and 50% amylopectin with molecular weight overlay. Sample prepared by Method 2.

Table 1 – Calculated molecular weight averages for amylose in Figures 1-3 from elution time of 20 minutes and onwards.

Sample (prep method) | MW (g/mol)
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0% amylose (1) | (1.5±0.2)x10^7
100% amylopectin | (1.2±0.1)x10^6
50% amylose (1) | (1.2±0.1)x10^6
50% amylopectin | (1.5±0.4)x10^6
50% amylose (2) | (1.5±0.4)x10^6
50% amylopectin | (1.5±0.4)x10^6

Conclusions
- Both cooking methods allowed complete dissolution of starch
- Cooking Methods 1 and 2 yielded different elution profiles
- Both cooking methods generally led to lower MWs for amylopectin (assuming elution after 20 minutes) than literature value (10^7 Da)
- Weak dRI signals may be contributing to underestimation of MWs

Future work
- Collection of amylose and amylopectin via organic flow field-flow fractionation to use as size standards
- ThFFF-MALS-dRI for quantification of amylose and amylopectin content
- Comparison of amylose and amylopectin content using standard iodine potentiometric titration method to determine accuracy of quantification of amylose and amylopectin content.
- Calculation of thermal diffusion and macro (normal) diffusion based on retention times

References