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Amylose and amylopectin in starch by asymmetric flow field-flow fractionation with multi-angle light scattering and refractive index detection (AF4–MALS–RI)

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ABSTRACT

The rheological and functional properties of starch are influenced by the size and molar mass distribution of the polymer, the ratio of amylose (AMY) to amylopectin (AMP), and branching characteristics. Asymmetric Flow Field-Flow Fractionation (AF4) was applied to fractionate five different maize hybrids of varying AMY:AMP ratio. When coupled to detection by multi-angle light scattering and refractive index (MALS–RI), it was possible to determine mass percentage and the average weight-average molar mass (M_w) without the need for calibration standards. Sufficient resolution of amylose and amylopectin was achieved by applying a gradient cross-flow on a 17 cm trapezoidal channel with a 350 μm spacer. The observed M_w ranged from about 2×10^5 to 4×10^5 for amylose and from 1×10^8 to 4×10^8 for amylopectin. The corresponding *z*-average root-mean-square radii (R_z) for AMP ranged from 145 to over 300 nm. Low recoveries from the AF4 channel were found to be due primarily to the focusing step. The calculated mass percent of AMY and AMP from integrated RI peak areas agreed well with nominal values for the individual starch hybrids. Both qualitative and quantitative data were reproducible. The results show the AF4–MALS–RI method to be well suited for routine molecular characterization of starch.

1. Introduction

In addition to being the most common source of digestible carbohydrate in the diet, starch is a naturally abundant, non-toxic, relatively inexpensive raw material. Starch and starch-derived materials find use in a variety of industrial applications including foods and beverages, pharmaceuticals, household products, cosmetics, paper and packaging, etc., where they may be employed as adhesives, absorbents, encapsulates, additives or binders. Starch and modified starch materials also show promise as low-cost replacements for synthetic polymers in some applications. Starch consists of large polydisperse glucose homopolymers of amylopectin (AMP) and amylose (AMY) that are arranged in crystalline and amorphous regions in granules within plant cells. The rheological and functional properties of starch are influenced by the ratio and average molar masses of these macromolecular constituents. Studies of AMP have shown it to be a highly branched structure containing a mixture of (1 → 4)-α-D-glucose and (1 → 6)-α-D-glucose linkages with molecular weights that can reach up to 10^8. AMY is more linear, consisting primarily of long chains of (1 → 4)-α-D-glucose linked residues and molecular weights in the 10^5 to 10^6 range (Aberle et al., 1994; Whistler and BeMiller, 1997). The AMY:AMP ratio differs among starches; levels of AMY and AMP in normal corn (*Zea mays* L.) starch are 25–28 and 72–75%, respectively. However, the starches of some mutant plants contain either an increased AMY content (e.g., high amylose or amylostarch with up to 70% AMY) or an increased AMP content (e.g., waxy starch with 99–100% AMP). The different AMY:AMP ratios of these starches lead to variations in granular structure, physicochemical properties and quality of end-use products. For example, the AMY content of rice (*Oryza sativa* L.) starch ranges from 0 to 30% (w/w). Cooked rice texture and rice starch functional properties are reported to be primarily impacted by AMY content (Bhattacharya et al., 1982). However, evidence is building that variations in other aspects of starch are also main determinants of cooking and processing quality, such as the molar mass of AMY, the weight- and molar-based distributions of degree of polymerization

Abbreviations: AF4, asymmetric flow field-flow fractionation; FFF, field-flow fractionation; AMP, amylopectin; AMY, amylose; DMSO, dimethylsulphoxide; MALS, multi-angle light scattering; M_w, weight-average molar mass; M_w/M_n, polydispersity; $\langle r_g^2 \rangle$, mean-square radius, gyration radius; R_z, *z*-average root-mean-square radius; RI, refractive index; SEC, size-exclusion chromatography; kDa, kiloDalton.

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(DP) of AMY, and the fine structure of AMP (Bergman et al., 2004; Chen and Bergman, 2007; Tsai and Lii, 2000; Umemoto et al., 2002; Vandeputte et al., 2003a,b). It is known that the physical characteristics of starch can vary with botanical source, geographical origin, crop year and environmental conditions. Therefore, it is important to be able to determine the values of certain quality indicators, AMP:AMY ratio and molar mass distribution, for example.

Since many of the applications of starch involve dissolution or dispersion in aqueous systems, analytical investigation of starch properties in aqueous solution is especially relevant for understanding structure–function in these systems (Fishman and Hoagland, 1994). However, there are difficulties associated with the separation and characterization of the macromolecular components of starch in aqueous systems. These include, but are not limited to, solubilization, degradation, and the potential for aggregation and retrogradation. Dissolution procedures should dissolve all of the starch material without degradation and checks need to be made to ensure that neither aggregation nor retrogradation occurs. Any viable separation technique has to be able to provide resolution over a broad molar mass range without the loss of significant amounts of material or changes induced by the separation method itself (Gidley et al., 2010).

A dissolution method whereby starches are first dissolved in dimethylsulfoxide (DMSO), precipitated with ethanol, followed by dissolution in water with the aid of microwave heating under pressure, has been shown to provide nearly complete solubilization without degradation under optimized conditions (Bello-Perez et al., 1996, 1998; Fishman and Hoagland, 1994; Fishman et al., 1996; Roger et al., 1999, 2001; Rolland-Sabate et al., 2007; Tetchi et al., 2007). This dissolution technique was applied earlier for the preparation of starch solutions for subsequent analysis by size-exclusion chromatography (SEC). While SEC is well established as a method for the analysis of polymers, problems associated with its use for very large macromolecules such as AMP (You et al., 2002) can include exceeding the exclusion limit of the column packing, irreversible interaction with the column and subsequently low recovery, and shear degradation (Cave et al., 2009; Gidley et al., 2010). For this reason, field-flow fractionation (FFF) methods have been investigated for the separation of intact starch polysaccharides. In the first investigation applying flow FFF to starch (Roger et al., 2001), corn starches of varying amylose content were separated on a symmetrical 30 cm channel equipped with a frit outlet, the purpose of which was to reduce the effect of sample dilution and increase detection signal to noise ratio.

In this paper, we report on a practical application of asymmetric flow field-flow fractionation with multi-angle light scattering and refractive index detection (AF4–MALS–RI) for the separation and characterization of AMY and AMP in commercial corn starch using water as eluent. AF4 is a sub-type of the larger family of FFF techniques (Giddings, 1993) and offers the advantage of an open-channel separation without shear degradation and/or column adsorption effects that can arise with SEC. Unlike SEC, resolution can be controlled by varying the cross-flow. Once sufficient resolution is achieved, detection by MALS–RI enables calculation of the mass percent of AMY and AMP and determination of weight-average molar mass (M_w) and root-mean-square radii (R_z) without the need for calibration standards. AF4 has been applied to the separation and characterization of starch previously (Kim et al., 2007; Roger et al., 2001; Van Bruijnsvoort et al., 2001; You et al., 2002). Resolution could potentially be improved by using an asymmetric channel of enhanced design and flow control. Higher resolution would enable more reliable molecular characterization and differentiation of cultivars where variations may be small. The aim of the present study was to evaluate the capability of AF4–MALS–RI to resolve AMY and AMP for both qualitative and quantitative determination of AMY:AMP mass ratio, as well as other molecular characteristics for different starch hybrids.

2. Materials and methods

2.1. Asymmetric flow field-flow fractionation (AF4)–multi-angle light scattering (MALS)–refractive index (RI)

AF4 separation occurs in an open channel as shown in Fig. 1. Two plates are separated by a spacer that determines the channel thickness and breadth. The upper plate is impermeable, while the bottom plate is constructed of a porous frit. An ultrafiltration membrane covers the bottom plate and serves as the accumulation wall to prevent penetration of sample through the bottom of the channel. Asymmetric flow FFF differs from symmetric flow in that channel flow is constantly lost through the single porous wall of the channel, thus there is a decrease in longitudinal flow velocity along the channel, compensated in part by the trapezoidal shape of the channel spacer (Litzén and Wahlund, 1991). The inflow solvent is introduced at the channel inlet at a volumetric flow rate, V_{in}. This inflow rate is divided into the channel or outlet flow rate, V_c, and the cross-flow rate, V_x, such that $V_{in} = V_x + V_c$. The cross-flow is thus the excess flow created by limiting the channel outflow to the detectors so that $V_c < V_{in}$. The cross-flow velocity is zero at the upper wall and increases nonlinearly toward the accumulation wall (Wahlund and Giddings, 1987). This cross-flow exits the channel through the porous accumulation wall, creating a flow field perpendicular to the longitudinal flow and forcing analyte molecules downward. Under laminar flow conditions, the channel flow has a parabolic profile where the velocity is greatest at the center of the channel and lowest at the walls (Giddings, 1993). The downward force induced by the cross-flow is balanced by diffusional forces and differences in diffusion coefficients of analyte molecules are the basis for the technique's selectivity. Lower masses with higher diffusion coefficients establish a steady state closer to the center of forward flow and thus elute first, while higher masses traverse more slowly.

The retention time, t_r, of a polymer in field-flow fractionation is approximated by,

$$\frac{t_r}{t_0} = \frac{w^2 V_x}{6DV^0} \tag{1}$$

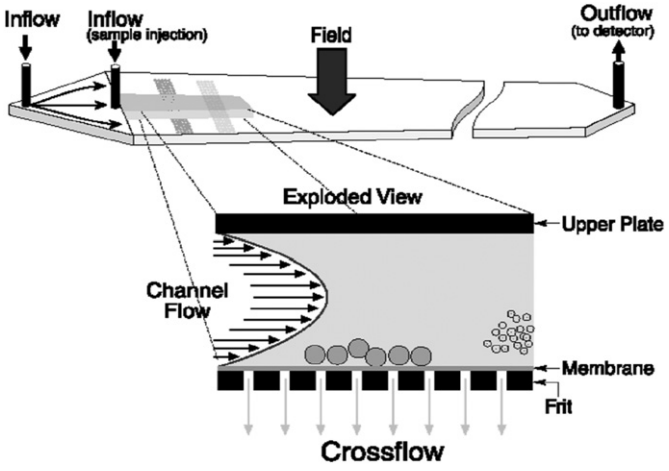

Fig. 1. Separation mechanism in AF4.