Characterization of Starch Cooked in Alkali by Aqueous High-Performance Size-Exclusion Chromatography[1]

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ABSTRACT

Commercial corn starches with different amylose-amylopectin (AMY-AMP) ratios were characterized using aqueous high-performance size-exclusion chromatography (HPLC-SEC). Starch (0.5%) was boiled in water for 10 min, autoclaved for 10 min, sonicated for 20 sec, and centrifuged for 10 min. Molecularly dispersed starch in the supernatant was separated into AMP and AMY using water as the mobile phase. AMP molecules have a wider range of hydrodynamic volumes and apparent molecular weights than do AMY molecules. Boiled and autoclaved starch was not molecularly dispersed in water. After sonication treatment, this cooked starch was molecularly dispersed in water without depolymerization; however, complete molecular dispersion was not achieved. Starch cooked with sodium hydroxide (NaOH) had increased water solubility, but starch was depolymerized or its effective diameter decreased at NaOH concentrations of 0.001 N (0.04% w/w) and greater. The AMP HPLC-SEC profile was more affected by sonication, NaOH, and CaO treatments than was AMY. CaO increased solubility and decreased starch apparent molecular weight or effective diameter, at levels as low as 0.005% CaO. An insoluble gel material was observed at 0.08% CaO; it increased with increasing CaO levels. Solubilized starch cooked with CaO consisted mostly of AMP and a starch fraction with an elution volume between AMP and AMY. This aqueous HPLC-SEC technique permits characterization of water-soluble starch in food systems.

Information about the molecular character and proportion of amylose (AMY) and amylopectin (AMP) in starches from different varieties and grains of different maturity has been gained using low-pressure size-exclusion chromatography (SEC) (Praznik et al 1987b, Craig and Stark 1984). Changes in starch molecular weight during drum drying and extrusion cooking have also been studied using SEC (Colonna et al 1984). Separation on traditional (low-pressure) SEC columns, however, involves tedious fraction collection and chemical analysis that requires at least one day per sample to complete (Kobayashi et al 1985). The time required for low-pressure SEC limits the number and type of samples that can be analyzed. For instance, a large number of samples can't be prepared simultaneously without risk of starch degradation, nor can reduced starch solubility caused by retrogradation be easily monitored. However, high-pressure liquid chromatography (HPLC) with SEC analysis gives separations similar to those from low-pressure columns but in less time. Takagi and Hizukuri (1984) used TSK-PW columns to separate AMY from potato and lily starches. Kobayashi et al (1985) separated and quantified AMY and AMP using μ-Bondagel columns. Structural and chemical properties of rice, potato, and tapioca AMY have also been evaluated and related to their functionality in foods (Takagi and Hizukuri 1984, Takeda et al 1986).

SEC separates molecules on the basis of their effective diameter and molecular weight (MW). However, the elution time of a polymer can be related to its MW when suitable standards are available. Although starch MW standards are not available, pullulan (α-1,6 linked trimaltose polymer) and dextran (95% α-1,6 linked, 5% α-1,3 linked polyglucan polymer) standards have been used to estimate starch MW (Takagi and Hizukuri 1984, Kobayashi et al 1985). Praznik et al (1987a) reported that pullulans had the same migration behavior in an aqueous system as did debranched starch molecules. Treatment effects that result in different SEC chromatograms can be the result of actual MW changes, or changes in molecular conformation. In the absence of MW measuring devices, treatment effects can be viewed as changes in apparent MW.

Preparation of starch for SEC or HPLC-SEC has proven difficult. Kobayashi et al (1985) used dimethyl sulfoxide (DMSO) to solubilize starch, and Takagi and Hizukuri (1984) solubilized AMY with NaOH before diluting with water. Unfortunately, starch has different molecular conformations and behaviors depending upon its solvent (Young 1984), and alkali can depolymerize starch (Kervinen et al 1985). Also, functionally active starch in food systems is either solubilized in water or is part of a hydrated gel. Methods need to be developed to characterize starch in water without using alkali to solubilize the starch. This is especially important if techniques are to be developed to analyze starch extracted from alkaline-processed food products (i.e., tortillas), where alkali solubilization of starch would be inappropriate.

The objectives of this work were to molecularly disperse starch in water and to develop HPLC-SEC techniques to study the apparent MW profile of this material. In addition, the apparent MW profiles of corn starch with different proportions of AMP and AMY, and the effects of alkali (NaOH and CaO) on the HPLC-SEC profile and solubility of corn starch were studied.

MATERIALS AND METHODS

HPLC-SEC Equipment and Procedures

A 0.5% starch suspension was prepared by bringing 0.5 g of starch and 1.0 ml of methanol (starch hydration aid) to a volume of 100 ml with water. Samples (10 ml) of this suspension were gelatinized by placing a test tube in boiling water for 10 min and autoclaving for 10 min. After the suspension had cooled and equilibrated to 55° C in an oven (30 min), the suspension was further dispersed by an ultrasonic processor (Biosonik II, Bronwill Scientific) for 20 sec. The solution was then centrifuged for 10 min at 3,400 $\times g$, and the supernatant was filtered through a 5-μm nylon filter. The dispersed starch was diluted with water and allowed to equilibrate at 55° C before HPLC-SEC analysis.

Starch solution (25 μl) was injected into four Shodex Ionpak S-800 series styrenedivinylbenzene columns connected in series. The four columns included the S-806/S (estimated exclusion limit [EEL] = 5×10^7 MW), S-805/S (EEL = 5×10^6 MW), S-804/S (EEL = 5×10^5 MW), and the S-803/S (EEL = 5×10^4 MW). The columns were maintained at 80° C. The water mobile phase was pumped with a Beckman-110B HPLC pump operating at 1.0 ml/min. The columns were connected to a Waters Associates model 410 refractive index detector set at sensitivity 128; the detector cell temperature was maintained at 50° C. Data were collected and peaks integrated using an Apple IIe with Adalab hardware and Chromatochart software (Interactive Microwares, State College, PA). The amount of starch passing through the column was determined by collecting 2.0-ml fractions and measuring starch (glucose × 0.90) by the phenol-sulfuric acid

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method (Dubois et al 1956). Pullulan MW standards (853,000 to 5,300) from Showa Denko K.K. (Tokyo, Japan) were dissolved in water as specified by the manufacturer and injected into the HPLC-SEC system. A standard curve was calculated using retention time and log MW.

Samples and Treatments

Corn starch with differing AMP-AMY ratios was obtained from American Maize-Products Corporation (Hammond, IN). Amylose contents of these starches were supplied by American Maize-Products and confirmed using iodine colorimetric AMY tests outlined by Knutson (1986) and Juliano (1971). The HPLC-SEC profiles of corn starch containing 100, 75, 47, and 25% AMP were studied with the HPLC-SEC system.

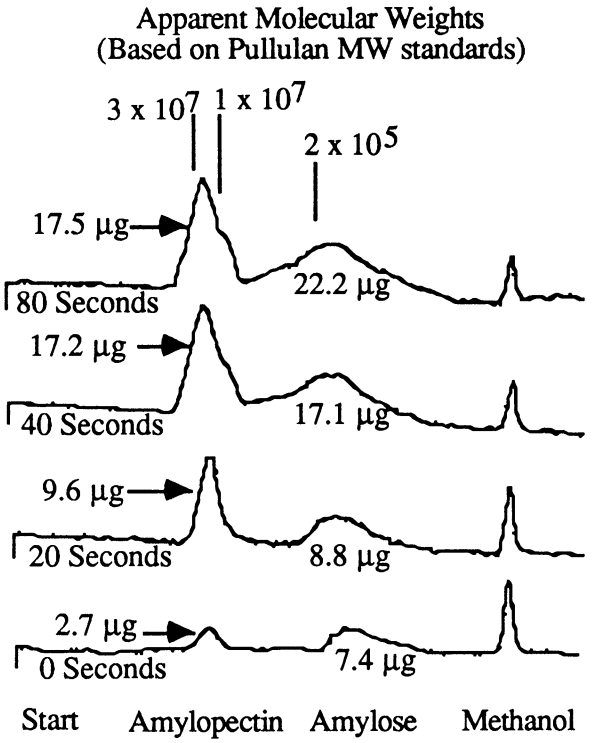

Fig. 1. The effect of sonication on the solubility of starch (53% amylose, 43 µg).

TABLE I
Water Solubility of Corn Starches Given Different Sonication and Alkali Treatments[a]

Treatment/ Sample	% of Total Amylopectin	% of Total Amylose	% of Total Starch
Sonication			
10 sec	14	32	23
20 sec	48	38	43
40 sec	86	74	80
80 sec	88	96	92
Corn starches			
30% AMP	42	20	26
47% AMP	49	40	44
75% AMP	61	43	49
100% AMP	48	N/A	48
NaOH-cooked starch			
No NaOH	48	39	43
0.001 *N*	66	50	57
0.048 *N*	94	64	78
0.091 *N*	77	39	56
CaO-cooked starch			
No CaO	45	39	42
0.08% CaO	90	25	56
0.50% CaO	85	19	50
1.06% CaO	71	12	40

[a] The 47% amylopectin (AMP) corn starch was sonicated for 20 sec, unless otherwise indicated.

The effect of ultrasonic waves on starch suspensions was determined by sonicating 53% AMY, 47% AMP starch for 0–80 sec in 10-sec intervals. The effect of NaOH on starch solubilization and its HPLC-SEC profile was tested by adding 1 *N* NaOH to the 10-ml starch suspensions before boiling. The concentration of NaOH in the starch suspension ranged from 0 to 0.091 *N* (pH 7–13). The effect of CaO on starch was similarly tested by adding CaO to the 10-ml starch suspension before boiling. Solutions with CaO concentrations of 0–1% were prepared (pH 7–12).

RESULTS AND DISCUSSION

Sonication

Starch prepared for HPLC-SEC must be molecularly dispersed, but starch is usually not molecularly dispersed in water without the addition of alkali. Zorina and El'piner (1963) showed that ultrasound can depolymerize dextran. It was conjectured that a short ultrasonic treatment would molecularly disperse cooked starch without causing depolymerization.

Mild sonication increased the water solubility of starch, although extensive sonication appeared to depolymerize amylopectin (Fig. 1). Szent-Gyorgyi (1933) also reported that ultrasound depolymerizes starch as measured by falling viscosity. The apparent MW range of both AMP and AMY increased with increasing sonication up to 80 sec; sonication for >80 sec substantially altered the HPLC-SEC profile. In the AMY fraction, lower apparent MW material was the first to be solubilized. Meyer et al (1949) found that lower apparent MW fractions of AMY are more easily solubilized in water. Ultrasonic vibrations disrupted granule integrity, which more fully dispersed both AMY and AMP. In addition, sonication broke up rapidly forming AMY aggregates, thus slowing retrogradation. Chromatograms of DMSO-solubilized starch presented by Kervinen et al (1985) suggest that starch aggregates were not fully dispersed. Even in DMSO, a better starch solvent than water, a sonication treatment would increase the solubility of starch. Sonication, however, allows the use of water for HPLC-SEC; water is a more appropriate solvent for the study of starch in food systems. Approximately 43% of the total starch, at neutral pH, was solubilized by a 20-sec sonication (Table I). Actual solubility depends on the type and origin of the starch, and the cooking treatment. Gelatinized starch not solubilized by sonication is unlikely to be solubilized in a food product.

Pullulans with known MWs (Showa Denko K.K., Tokyo Japan) were evaluated in the HPLC-SEC system. The elution times of these pullulans, which had monodispersed symmetrical peaks, were used to calculate starch apparent MW (Fig. 2).

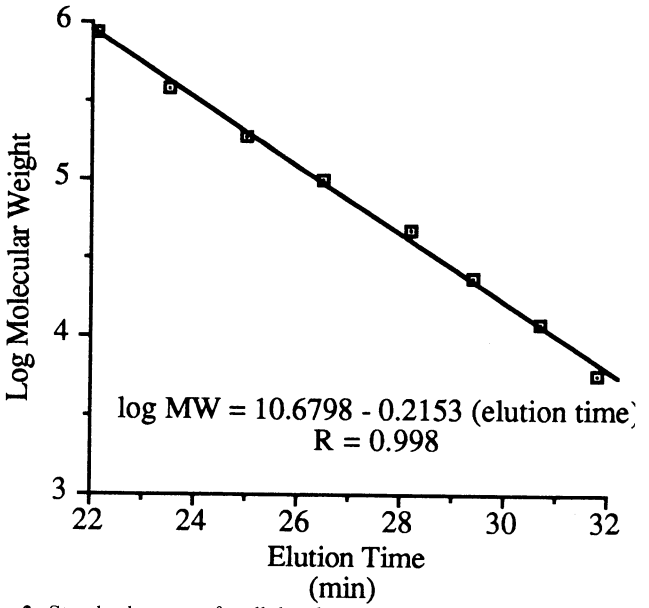

Fig. 2. Standard curve of pullulan log molecular weight versus high-performance size-exclusion chromatography elution time.

Starch Type

HPLC-SEC characterized the apparent MW solubility profiles of starches with different AMP and AMY ratios (Fig. 3). Molecules that eluted first from the HPLC-SEC columns had the largest hydrodynamic volume and apparent MW. Hydrodynamic volume and apparent MW then decreased logarithmically with elution time. Hence, AMP molecules span a larger range of apparent MWs than AMY molecules, even though AMY peaks are wider than the AMP peaks.

The average apparent MW of AMY increased as the percentage of AMY in a starch decreased. The AMP peaks of corn starches were not symmetrical. Some intermediate apparent MW materials between AMP and AMY were present in these starches. Similar AMP "tailing" of sorghum starches was observed by Craig and Stark (1984). The relative water solubility of AMP and AMY does not conform to the ratio of these components in native starch (Table I). Less AMY was solubilized than AMP. Meyer et al (1949) noted that pure amylose is far less water soluble than the amylose in an AMY-AMP mixture. The percentage of AMY solubilized decreased from 43 to 20% as the total percentage of AMY in starch increased from 30 to 75%. Total water solubility of the starches, without additional treatment, did not exceed 50%.

Alkali-Cooked Starches

Starch molecules underwent molecular changes, probably depolymerization, when cooked with NaOH at concentrations as low as 0.001*N* (Fig. 4). Over a period of 5 hr, $10^{-6}N$ NaOH appeared to depolymerize amylopectin. AMP was affected more readily than AMY. Increasing amounts of NaOH increased the total quantity of solubilized starch fractions; AMP was solubilized more readily by NaOH than AMY (Table I). The observed formation of insoluble aggregates in the cooked starch solution at 0.09*N* NaOH, however, decreased total starch solubility from 78% (0.048*N* NaOH) to only 56%. Kervinen et al (1985) monitored the apparent MW profile of wheat starch extruded with alkali. Although separated with less resolution, these workers presented chromatograms with similar patterns, attributing them to starch depolymerization. The apparent molecular changes of starch molecules in food products cooked in alkaline solutions can be monitored by aqueous HPLC-SEC.

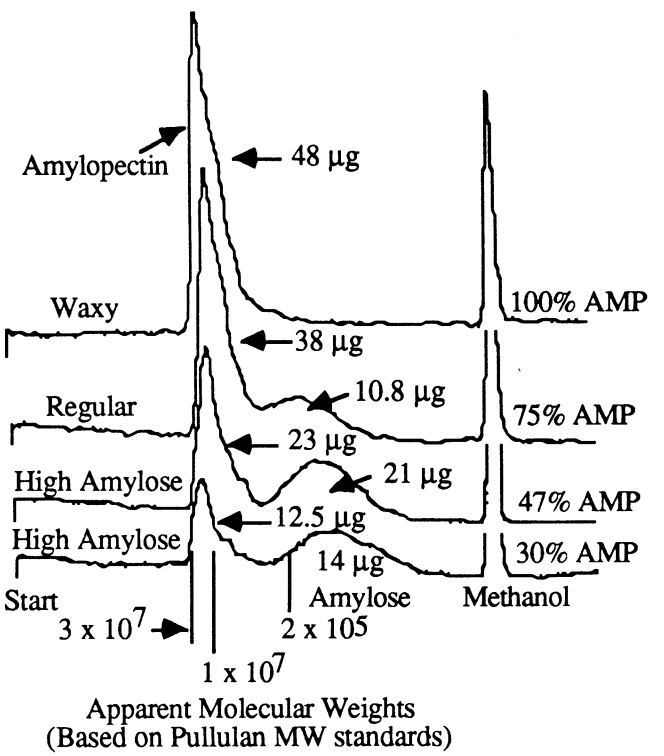

Fig. 3. Characterization of corn starches (100 µg) with different amylose-amylopectin ratios (20 sec sonication time). **AMP** = amylopectin.

Starch cooked in CaO contained more soluble AMP than starch cooked without CaO. An AMY gel, however, formed on the bottom of centrifuge tubes containing CaO cooked starch. Therefore, the amount of soluble AMY was substantially reduced in these samples (Fig. 5 and Table I). At 1.06%, w/w, of CaO, extensive AMY gelation trapped AMP, reducing the solubility of both AMY and AMP. Although more study is necessary, the formation of Ca-starch derivatives, Ca-AMY cross-linking, conformational changes, or ordinary alkaline depolymerization of starch probably contributed to the unusual chromatography patterns. The formation of a highly insoluble AMY fraction in CaO cooked starch may account for the rapid firming of alkaline-cooked corn masa and tortillas.

HPLC-SEC System Limitations

The HPLC-SEC system used for this study has a number of limitations. Current pullulan and dextran MW standards are suitable for approximating MWs up to 10^6. Separate MW

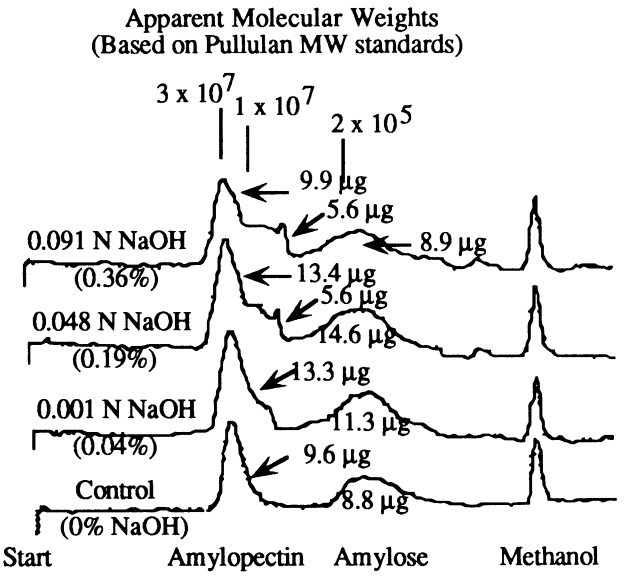

Fig. 4. High-performance size-exclusion chromatographic profiles of starch (53% amylose, 43 µg) cooked with NaOH (20 sec sonication time).

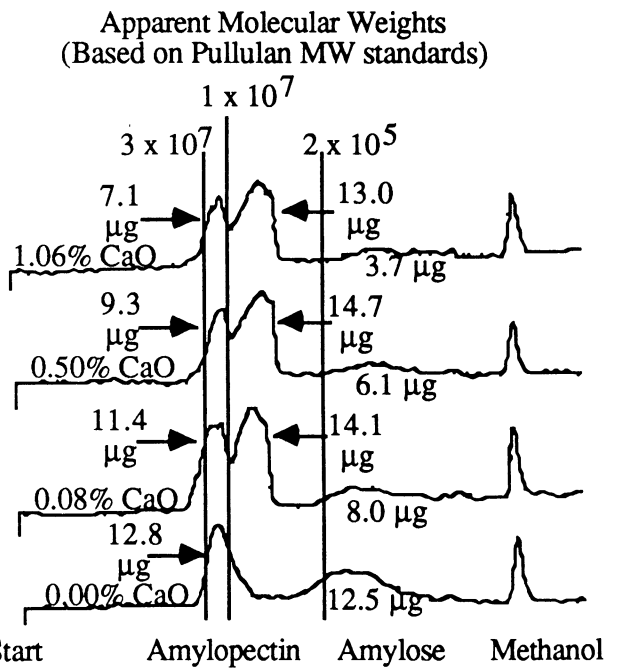

Fig. 5. High-performance size-exclusion chromatographic profiles of starch (53% amylose, 60 µg) cooked with CaO (20 sec sonication time).

standards with hydrodynamic volumes similar to AMP and AMY are needed in the 10^6 to 10^8 MW range.

Certain negatively charged ions (i.e., Cl^-, but not OH^-) are not separated on the basis of their molecular size. These anions elute faster than if they were separated on the basis of molecular size alone, and Cl^- elutes at an apparent MW near that of AMY. Preparation and analysis of food samples and starches that contain anions require their removal. In addition, the columns bound some starch when new, but after a short equilibration time, starch eluted quantitatively. The columns can be effectively cleaned with dilute NaOH (pH 10).

New materials for HPLC columns will be developed, and these limitations will be eliminated. The real challenge is the development of techniques to appropriately solubilize starch prior to HPLC-SEC or other analyses.

SUMMARY

Sonication increased the solubility of starch in water and permitted apparent MW characterization of starches without the use of DMSO or alkaline conditions. Extended sonication began to alter the HPLC-SEC profiles of AMP and AMY. Starches with different proportions of AMY and AMP gave different aqueous HPLC-SEC solubility profiles. AMP molecules spanned a wider range of apparent molecular weights than AMY molecules. Both sodium hydroxide and calcium oxide, when cooked with starch, altered the elution volumes of AMY and AMP, and generally increased their solubilities. Sodium hydroxide appeared to rapidly depolymerize and increase the solubility of AMY and AMP at concentrations as low as 0.001 *N* (0.01%). Calcium oxide increased solubility of starch at levels as low as 0.005%. A fraction with an apparent MW between AMP and AMY was also formed. At CaO levels >0.08%, starch suspensions begin to gel, reducing solubility of both AMY and eventually AMP. This aqueous HPLC-SEC system allows for the characterization of water soluble starches in food systems, and enables researchers to monitor changes in starch structure that occur during food processing.

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