Differential Water Solubility of Corn and Sorghum Starches as Characterized by High-Performance Size-Exclusion Chromatography[1]

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ABSTRACT

The inherent water solubility properties of corn and sorghum starches were studied with high-performance size-exclusion chromatography (HPSEC). Corn starches containing <1, 25, 53, and 70% amylose (AMY), and sorghum starches containing <1, 17, 23, and 24% AMY were solubilized in water. Corn starch was solubilized at 85°C for 1 or 4 hr by traditional aqueous leaching procedures. Corn and sorghum starches were also solubilized at 65°C, 85°C, or boiled and autoclaved, and subsequently sonicated. After treatment, starch solutions were centrifuged and filtered in preparation for HPSEC analysis. Traditional aqueous leaching solubilized mostly AMY. Soluble AMY had a smaller hydrodynamic volume and hence a smaller apparent molecular weight as the starches increased in total AMY content. AMY was more soluble on a percentage basis from starch with less total AMY than from starch containing more AMY. Starch treated at different temperatures had an increased amount of starch solubilized and an increased apparent molecular weight of AMY at higher temperatures. Differential scanning calorimetry endotherms showed that crystalline regions from high AMY starch were not melted at 85°C, and as a result, these starches had lower HPSEC water solubilities. The endotherms showed that all sorghum starches melted below 85°C, yet AMY from sorghum starch with more total AMY was still less soluble. Hence, water solubility of AMY is governed by the crystalline melting behavior and total AMY content of the starch. Sorghum amylopectin (AMP), solubilized at 85°C, was separated into two populations. The AMP in cooked sorghum starches (120°C) was less soluble as the total sorghum AMP content increased. HPSEC AMP peaks from waxy starches show "tailing" in an area normally occupied by AMY molecules. HPSEC is a useful technique for characterizing the water-soluble components of native starch and starch-containing foods.

Characterization of starch by high-performance size-exclusion chromatography (HPSEC) has proven useful for the study of amylose structure, separation and quantification of amylose and amylopectin, and the characterization of starch treated with alkali (Takagi and Hizukuri 1984, Kobayashi et al 1985, Jackson et al 1988). Starch molecules must be soluble before HPSEC analysis. Many workers have utilized dimethylsulfoxide or alkali to solubilize starch (Takagi and Hizukuri 1984, Kobayashi et al 1985); whereas these are better solvents, starch is solubilized by water in food products. Recently, a technique was developed to solubilize starch molecules in water using ultrasonic waves prior to HPSEC analysis (Jackson et al 1988). However, for this technique to be appropriately applied to starch-containing food systems, the inherent water solubility of different starches needs to be studied and characterized.

The objective of this study was to solubilize starches containing different proportions of amylose (AMY) and amylopectin (AMP) in water of different temperatures and to study the inherent water solubility properties of these starches using HPSEC. Changes in starch solubility and HPSEC profiles caused by food processing can then be interpreted with respect to the inherent water solubility of untreated starches.

MATERIALS AND METHODS

Starch Samples

Commercially isolated corn starches containing <1, 25, 53, and 70% AMY were obtained from American Maize-Products Corp. (Hammond, IN). Laboratory-isolated waxy, heterowaxy (2 types), and nonwaxy sorghum starches from crosses made with very closely related kaffir sorghums (nonwaxy Tx3197 and waxy Tx615) were also used (Lichtenwalner et al 1978). These starches contained <1, 17, 23, and 24% AMY, respectively. Three of the four sorghum starches have at least one recessive waxy gene, which decreases the AMY content. Three recessive waxy genes produce kernels containing starch with no AMY (Lichtenwalner et al 1978).

AMY contents of sorghum starches were determined by a potentiometric procedure (Lichtenwalner et al 1978), and corn starch AMY values obtained from American Maize-Products were confirmed using colorimetric iodine tests outlined by Juliano (1971) and Knutson (1986).

Starch Solubilization and HPSEC Separation

Corn and sorghum starch suspensions (1%) were prepared by bringing 1.0 g of starch and 0.5 ml of methanol to a volume of 100 ml with water. The small amount of methanol helped to "wet" the starch, assuring good dispersion. Ten milliliters of this suspension was gelatinized by placing a test tube of the sample in boiling water for 10 min and autoclaving (120°C) for 10 min. After the suspension had cooled to 55°C in an oven, the starch was further dispersed by an ultrasonic processor (Vibra-cell model VC 40; Sonics & Materials, Danbury, CT) equipped with a 3-mm microtip, for 20 sec. The solution was centrifuged for 10 min at $3,400 \times g$, and the supernatant was filtered through a 5 μm nylon filter. The dispersed starch was next diluted with three parts water and allowed to equilibrate at 55°C before HPSEC analysis. Corn and sorghum starches were also solubilized in 65 and 85°C water, sonicated, centrifuged, and filtered. Starch treated at 65°C was not diluted before injection, whereas starch treated at 85°C was diluted with two parts water. The water solubility characteristics of corn starches treated by traditional aqueous leaching (Meyer et al 1949) at 85°C were also studied using HPSEC. Samples with different AMY contents were gently stirred at 85°C for 1, 2, 3, or 4 hr and were not sonicated before centrifugation and filtration.

Starch solution (25 μl) was injected into four S-series Shodex Ionpak columns (Showa Denko K.K., Tokyo, Japan) connected in series. These columns separate polysaccharides by molecular weight and effective diameter. Water was used as the mobile phase (flowing at 1 ml/min), and the separated compounds were detected using a refractive index detector (Waters model 410; Millipore Corp., Milford, MA). Data were collected and peaks integrated using an Apple IIe with Adalab hardware and Chromatochart software (Interactive Microwares, State College, PA). Samples were processed and analyzed in groups of four, and each series was replicated. The amount of starch passing through the column was determined by collecting 2.0-ml fractions (one fraction every 2 min) and measuring starch (glucose $\times$ 0.90) by the phenol-sulfuric acid method (Dubois et al 1956). Pullulan molecular weight (MW) standards (5,300–853,000) from Showa Denko K.K. were dissolved in water and injected into the HPSEC system. Gel permeation chromatography software (Interactive Microwares) was used to construct a standard curve ($r = 0.999$) to relate log MW

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and retention time. A standard curve was used to calculate the apparent MW of starch peaks (Jackson et al 1988). Solubility and MW trends were consistent between samples processed together. The percent soluble starch differed by <8% of the percentage solubility reported for identical samples processed at different times.

Gelatinization characteristics of 3-mg starch samples in excess water were determined by differential scanning calorimetry (DSC) with a Perkin-Elmer DSC-4 as outlined by Krueger et al (1987). The samples were scanned from 23 to 123°C at a scanning rate of 10°C/min; an empty sample pan was used as the reference and each sample was run in triplicate. Coefficients of variation ranged from 0.0 to 3.3 for the temperatures measured at endotherm peak start, endotherm maximum, and peak end.

RESULTS AND DISCUSSION

Valuable information about how food processing affects starch can be obtained using HPSEC. However, before interpreting HPSEC patterns from starch extracted from foods, the inherent solubility of native starch in water must be characterized. Also, by studying the solubility of native starch at different temperatures, data on how starch polymers become soluble in foods can be collected.

HPSEC separates molecules on the basis of their hydrodynamic volume, which is related to both its effective diameter and MW. Different HPSEC profiles can be the result of differences in actual MW or molecular conformation. In the absence of direct MW measurements, HPSEC profiles should be viewed as changes in apparent MW. The apparent MW of compounds separated on these HPSEC columns decreases logarithmically with increasing elution time (Jackson et al 1988).

Corn Starch Solubility After Aqueous Leaching

Gentle stirring of swollen starch granules in water maintained at or near starch gelatinization temperature solubilizes AMY with only slight solubilization of AMP (Meyer et al 1949, Schoch 1945). Figures 1 and 2 show the effect of starch type and solubilization time on the water solubility of starches gently stirred at 85°C for 1 and 4 hr, respectively. Increasing amounts of AMY were solubilized by aqueous leaching over time, except for waxy starch. Only a small amount of the remaining AMY was solubilized after 1 hr. Normal corn starch AMY was 23 and 29% solubilized after 1 and 4 hr, respectively. Schoch (1945) found 26% of AMY from corn starch was solubilized at 85°C after 1 hr, and Banks et al (1959) found that about 22% of the total AMY was leached from barley starch at 70°C.

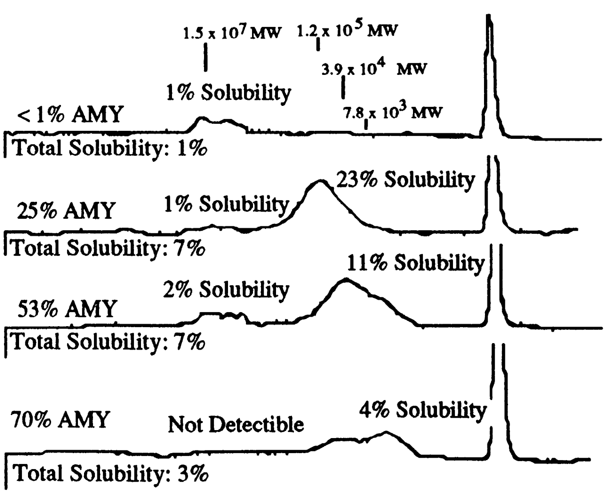

Fig. 1. High-performance size-exclusion chromatography solubility profiles of corn starch (1% solutions) extracted at 85°C by aqueous leaching for 1 hr. Data include total starch solubility, solubility of amylose (**AMY**) and amylopectin (**AMP**), and apparent molecular weights (**MW**).

Waxy starch, containing no AMY, was slightly soluble when leached at 85°C. But even after 4 hr of treatment, the solubility of AMP from waxy starch was similar to that of AMP from the other starches. The apparent MW of the AMP was 1.6×10^7.

AMY was the primary polymer solubilized by aqueous leaching. Soluble AMY from starch containing more total AMY had a smaller hydrodynamic volume, and hence, a smaller apparent MW than starch with less AMY. The average apparent MWs of AMY from 25, 53, and 70% AMY starches were 1.2×10^5, 3.9×10^4, and 7.8×10^3, respectively. The average apparent MW of AMY solubilized for 1 and 4 hr was not different. Taki et al (1988) reported that when starches containing the amylose-extender gene (*ae*) were solubilized with hot aqueous 1-butanol, the soluble AMY had a lower MW than soluble AMY from other starches. Also, Figures 1 and 2 show that the solubility of AMY from starch containing more total AMY was less soluble than from starches containing less AMY.

For individual starch polymers to become soluble, crystalline areas of the granule must be melted. Gelatinization data of starches in excess water, obtained from DSC, indicate that crystalline melting of high-AMY starches was not completed until temperatures exceeded 100°C (Table I). Therefore, starches with 53 and 70% AMY would not be fully solubilized by aqueous leaching at 85°C.

Aqueous leaching for 4 hr solubilizes <9% of the total starch, most of which is AMY. Water solubles from starch-containing food products, whose processing has not ruptured starch granules,

TABLE I
Differential Scanning Calorimetry Gelatinization Profiles of Corn and Sorghum Starches[a]

Starch Sample	Peak Start (°C)	Endotherm Maximum (°C)	Peak End (°C)
Corn			
70% amylose	62.3 (1.6)	92.2 (3.3)	106.4 (3.3)
53% amylose	62.6 (3.3)	74.8 (0.4)	104.6 (0.2)
25% amylose	61.8 (0.5)	71.4 (0.4)	80.1 (0.4)
<1% amylose	61.8 (0.9)	72.7 (1.2)	82.4 (2.0)
Sorghum			
24% amylose	69.0 (1.7)	74.5 (0.5)	83.6 (0.0)
23% amylose	68.6 (0.8)	74.4 (0.2)	83.1 (2.3)
17% amylose	70.8 (0.0)	76.2 (0.6)	84.5 (0.0)
<1% amylose	70.8 (0.0)	76.1 (0.4)	82.7 (0.0)

[a]Values are means of three analyses followed by coefficients of variation in parentheses.

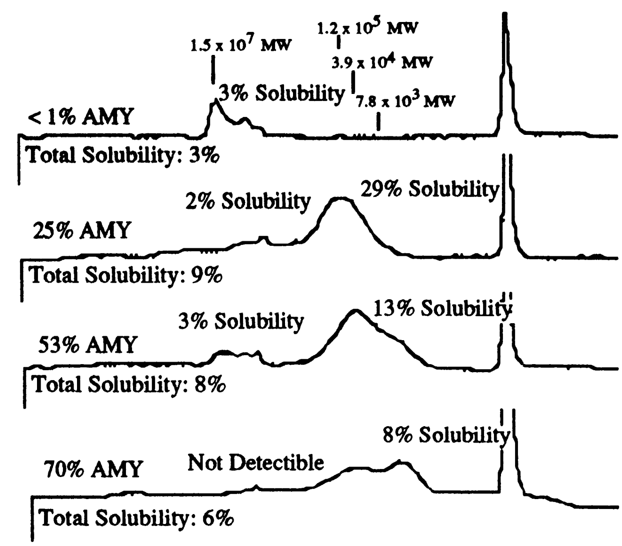

Fig. 2. High-performance size-exclusion chromatography solubility profiles of corn starch (1% solutions) extracted at 85°C by aqueous leaching for 4 hr. Data include total starch solubility, solubility of amylose (**AMY**) and amylopectin (**AMP**), and apparent molecular weights (**MW**).

will contain mostly AMY. This AMY will remain in solution or reaggregate depending upon the processing treatment and other food components.

Corn Starch Solubility After Sonication

Starch treated using more rigorous processing conditions, however, shows different solubility patterns. Starch that was extracted at 65° C, 85° C, or boiled and autoclaved, and subsequently sonicated, had a greater total starch solubility than starch that was not sonicated (Figs. 3–5). Sonication disrupts swollen granules, releasing both AMY and AMP from the granule. The sonicator used in this study was designed to treat small samples, and therefore solubilized more starch than a model used previously (Jackson et al 1988). While AMP is more difficult to remove from the granule than is AMY during aqueous leaching, AMP is generally more soluble than AMY once the granule is disrupted.

Starches solubilized in water for 10 min at 65°C were not soluble, except for waxy starch (Fig. 3). Granules swollen at 85° C and disrupted by sonication were substantially more soluble than

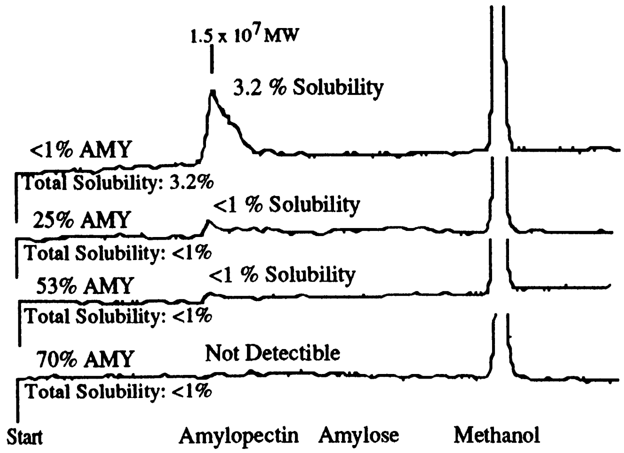

Fig. 3. High-performance size-exclusion chromatography solubility profiles of corn starch (1%) extracted at 65° C for 10 min and sonicated 20 sec. Data include total starch solubility, solubility of amylose (**AMY**) and amylopectin (**AMP**), and apparent molecular weights (**MW**).

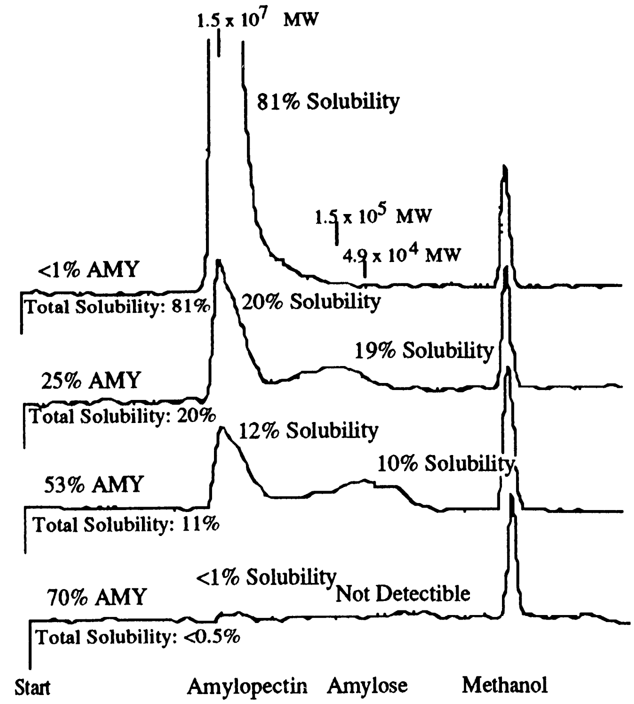

Fig. 4. High-performance size-exclusion chromatography solubility profiles of corn starch (1%) extracted at 85° C for 10 min and sonicated 20 sec. Data include total starch solubility, solubility of amylose (**AMY**) and amylopectin (**AMP**), and apparent molecular weights (**MW**).

when treated at lower temperatures (Fig. 4). For example, most of the waxy starch was solubilized at 85° C. AMP from starch with 25 and 53% AMY was partially solubilized (20.3 and 11.9% soluble, respectively). Complete starch crystal melting did not occur until temperatures of 104.6 and 106.4° C were obtained for 53 and 70% AMY starches, respectively (Table I). Thus, the high-AMY starches, which had higher melting temperatures, were less soluble than 25% AMY starch and waxy starch which both had melting temperatures below 85° C. The average apparent MW for AMY in starches containing 25 and 53% AMY was 1.5×10^5 and 4.9×10^4, respectively. The apparent MW of AMP was 1.5×10^7.

Boiled, autoclaved, and sonicated starches were 54–81% soluble (Fig. 5). AMY solubility decreased with increasing total AMY. AMP from waxy starch was not more soluble than the AMP solubilized at 85°C. AMP from waxy starch also shows the characteristic nonsymmetrical "tailing" into apparent MWs normally thought to contain AMY molecules (Craig and Stark 1984, Jackson et al 1988). Solubility of AMP from regular starch was slightly higher than AMP from waxy starch and from 70% AMY starch.

The apparent MW of AMY solubilized by autoclaving was higher than the AMY solubilized at 85° C. Average apparent MWs of AMY for starches containing 25, 53, and 70% AMY were 5.2×10^5, 1.7×10^5, and 8.3×10^4, respectively. The apparent MW of AMP did not change. Apparent MWs of AMY solubilized by sonication were greater than those solubilized by aqueous leaching. The lower MW AMY of a particular starch apparently is solubilized at lower temperatures than higher MW AMY. Ellis and Ring (1985) were able to obtain higher MW pea and potato AMY (obtained using *n*-butanol) at higher leaching temperatures. Meyer et al (1949) also reported that lower MW AMY from regular corn starch was solubilized at lower temperatures than was higher MW regular corn AMY.

Sorghum Starch Solubility After Sonication

None of the sorghum starches were soluble at 65° C. As Table I

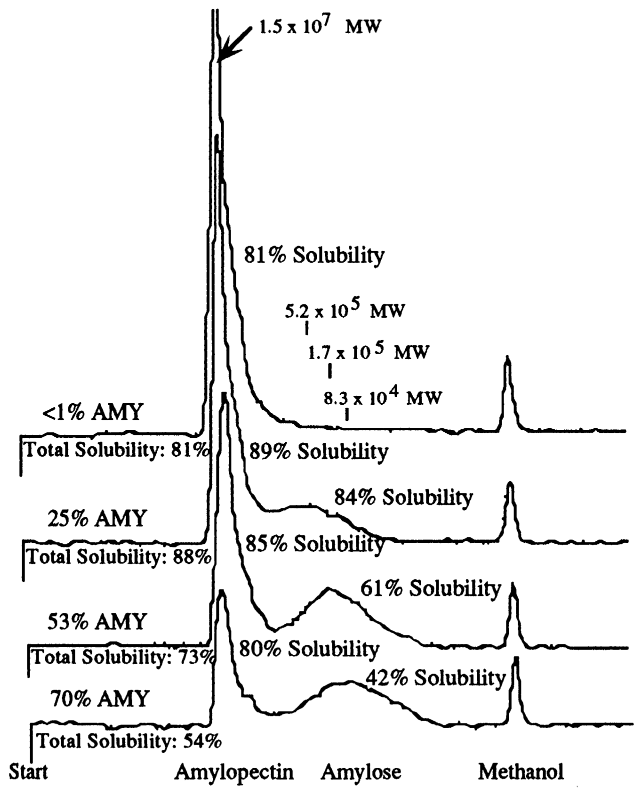

Fig. 5. High-performance size-exclusion chromatography solubility profiles of corn starch (1%) boiled for 10 min, autoclaved for 10 min, and sonicated 20 sec. Data include total starch solubility, solubility of amylose (**AMY**) and amylopectin (**AMP**), and apparent molecular weights (**MW**).

indicates, initial crystalline melting of sorghum starch did not occur until temperatures exceeded 68°C. The sorghum starches' initial melting temperatures were nearly 7°C above that of the corn starches.

Sorghum starches that were either solubilized at 85°C or boiled and autoclaved before sonication (Figs. 6 and 7), yielded some characteristics similar to the corn starches. As the total AMY content of sorghum starches treated at 85°C (Fig. 6) increased, the AMY solubilized from those starches had smaller hydrodynamic volumes. The apparent MW of AMY decreased from 2.9×10^5 for 17% AMY starch to 1.3×10^5 for 24% AMY starch. Also, AMY from 24% AMY sorghum starch was less soluble than AMY from starches with less total AMY.

The sorghum AMP solubilized at 85°C had two distinct populations of AMP, with apparent MWs of 1.2×10^7 and 5.6×10^6, respectively (Fig. 6). The increased starch solubility of boiled and autoclaved starches either masks these two populations, or boiling and autoclaving solubilizes other AMP molecules more completely.

As with corn starches, the AMY apparent MW was higher in the autoclaved starch (Fig. 7) than in sorghum starch solubilized at 85°C (Fig. 6). Autoclaved soluble AMY apparent MWs decreased from 3.6×10^5 to 2.4×10^5 for 17% AMY and 24% AMY starch, respectively. Because of the AMP tailing associated with both waxy AMP and AMP from some regular sorghum starches (Craig and Stark 1984), quantitative separation of AMP and AMY is difficult. The solubility of AMP is underestimated, whereas the apparent solubility of AMY is overestimated.

Starch solubility increased with increasing AMP for starch treated at 85°C. Interestingly, however, the solubility of AMP in autoclaved starch decreased with increasing AMP content (Fig. 7). In starch containing small amounts of AMY, a small amount of AMP tailing greatly inflates the apparent solubility of AMY. Waxy and heterowaxy starches either contain AMP of lower apparent MW, or the AMP in these starches is less soluble than AMP from starches without the recessive waxy gene. Taki et al (1988) suggested that AMP from waxy starches was different from AMP from normal and high-AMY starch on the basis of the fractionation behavior of AMP from waxy (*wx*) and waxy-amylo (*wx ae*) starch in aqueous 1-butanol compared to that of normal and high-amylose (*ae*) starch. When examining water solubility of the corn starches, it seems anomalous that AMP solubility increases with increasing AMP content, yet AMP from 100% AMP (waxy) starch is less soluble than AMP from 75% AMP starch. In addition, the AMP solubility of sorghum starch clearly shows a trend toward decreasing AMP solubility with increasing waxy character. We concur with Taki et al (1988) that AMP from waxy starches is different in its solubility behavior than that found in normal starches.

DSC data from the sorghum starch is not sufficient to explain the solubility profiles of the starches at 85°C (Fig. 7). The DSC data suggest that the thermal behavior of these starches is similar; i.e., the starch melted below 85°C. Whereas the solubility of corn starches at 85°C is due, in part, to the crystalline melting temperature, the absence of differences in the sorghum starch DSC data suggests that the actual AMY content may affect the solubility of starch. AMY within granules may be more tightly associated with AMP when more total AMY is present. Alternately, molecularly solubilized AMY may aggregate more quickly in the presence of larger amounts of AMY molecules. Hence, the

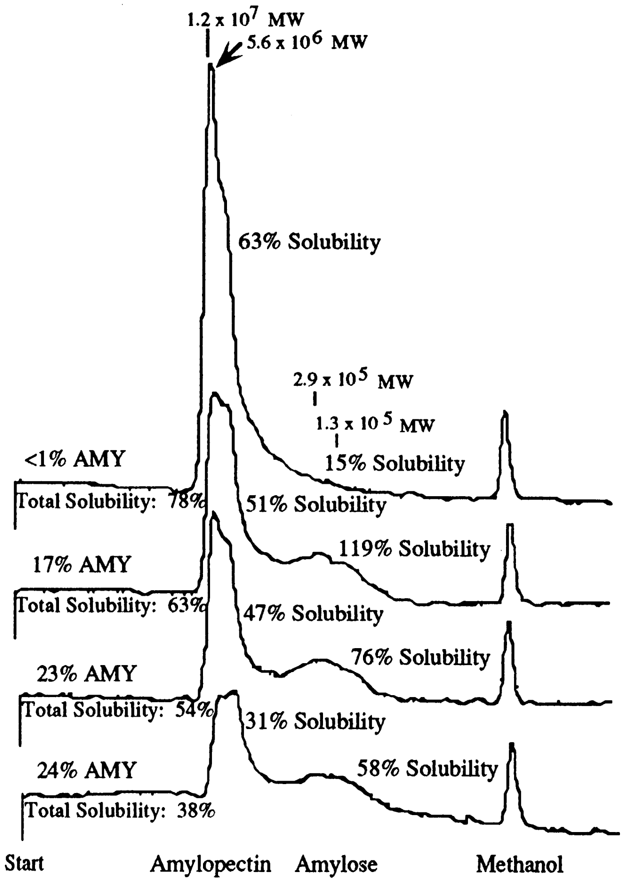

Fig. 6. High-performance size-exclusion chromatography solubility profiles of sorghum starch (1%) extracted at 85°C for 10 min and sonicated 20 sec. Data include total starch solubility, solubility of amylose (**AMY**) and amylopectin (**AMP**), and apparent molecular weights (**MW**). AMY solubility includes low-MW AMP. The amount of soluble low-MW AMP from waxy starch (<1% AMY) is presented.

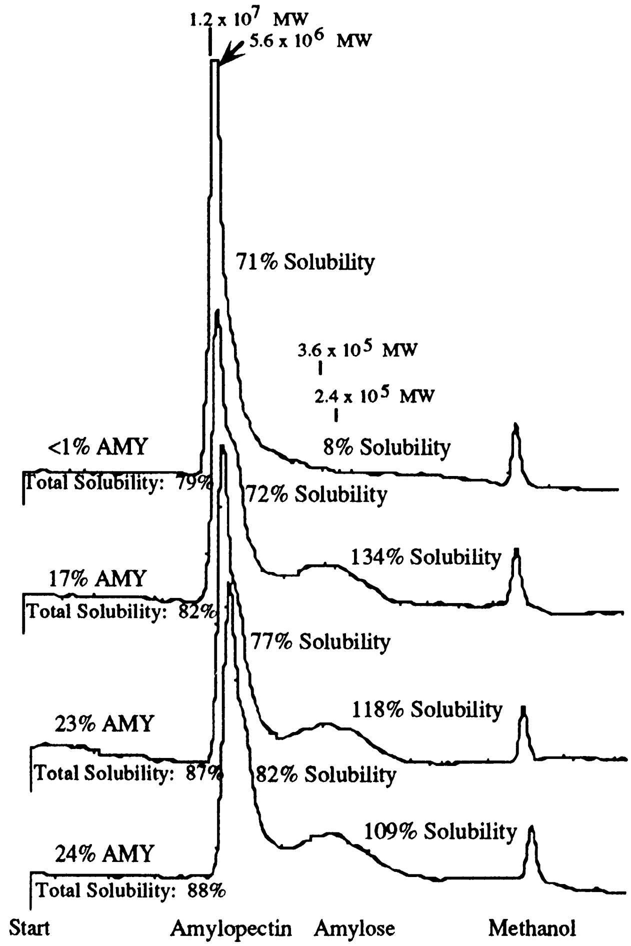

Fig. 7. High-performance size-exclusion chromatography solubility profiles of sorghum starch (1%) boiled for 10 min, autoclaved for 10 min, and sonicated 20 sec. Data include total starch solubility, solubility of amylose (**AMY**) and amylopectin (**AMP**), and apparent molecular weights (**MW**). AMY solubility includes low-MW AMP. The amount of soluble low-MW AMP from waxy starch (<1% AMY) is presented.

apparent AMY solubility would be reduced when measurements are taken.

Summary

Starch in food systems is hydrated and partially solubilized in water during preparation. Understanding the characteristics that govern starch water solubility will increase our knowledge of starch functionality in foods. Swollen starch granules, if not broken, will have mostly AMY leached from them. If these swollen granules are sheared, both AMP and AMY become soluble. In general, AMP is more soluble than AMY. Soluble AMY in starches with larger percentages of total AMY have a smaller hydrodynamic volume, and hence a smaller apparent MW than starches with less total AMY. Also, AMY from starches with less total AMY is more soluble than AMY from starch with more total AMY. AMP from waxy starches does not give symmetrical HPSEC peaks, i.e., AMP tailing represents starch molecules with smaller hydrodynamic volumes and lower apparent MWs. Also, AMP from fully cooked waxy starch is not as water soluble as AMP from other starches. As the "waxy character" (AMP content) of cooked sorghum starches increased, the water solubility of those starches decreased. AMP from starches with recessive waxy gene(s) are different from AMP's from other starches.

The solubility of starch molecules at temperatures below autoclaving is related to the degree of crystalline region melting and AMY content. AMY solubilized at lower temperatures has a smaller average apparent MW than AMY from fully cooked starch. The solubility and composition of starch initially solubilized in food products is probably closely related to the type of starch, degree of granule melting, and granule integrity remaining after processing. This study characterized the water solubility of some native starches; future work characterizing starch from food products will be interpreted with respect to both inherent and process-induced changes in starch solubility.

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