Effects of Single-Screw Extrusion Cooking on Starch as Measured by Aqueous High-Performance Size-Exclusion Chromatography[1,2,3]

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ABSTRACT

The water solubilization and apparent molecular weights of starch polymers from extruded corn starch and extruded decorticated sorghum flours were characterized by aqueous high-performance size-exclusion chromatography (HPSEC). Corn starch (25% amylose) was extruded at moisture contents ranging from 11.3 to 32.2%. Decorticated sorghum flours (12, 22, and 27% amylose) were extruded at 18, 32, and 45% moisture. In preparation for HPSEC analysis, extruded corn starch was solubilized in water for 10 min at 35°C. Extruded decorticated sorghums were also treated at 35°C, or boiled 10 min and autoclaved 10 min to solubilize starch. Although no dextrinization of the corn starches was evident, the HPSEC-determined water solubility increased with decreasing extrusion moisture. Decreased water solubility of corn starch, however, was caused by extrusion below 14% moisture content. Water solubility of starch in sorghum extrudates generally decreased as the amylose content increased. Two populations of amylopectin from sorghum were detected by HPSEC analysis. Because raw and extruded sorghum flours still exhibited differences in starch solubility even after both samples were autoclaved in excess water, extrusion cooking modifies starch polymers differently than cooking in excess water.

Extrusion is a cooking and shaping process designed to give unique physical and chemical functionality to food materials. High pressures and temperatures are common in cooking extruders, thereby causing changes in the physical and chemical properties of extruded starch (Linko et al 1981).

The effects of extrusion on water solubility and water absorption of starch have been studied extensively (Anderson et al 1969, Colonna and Mercier 1983, Colonna et al 1984, Gomez and Aguilera 1984). These reports indicate that with decreased extrusion moisture and higher temperatures, water solubility of extruded material is greater than that of either raw material or material extruded at higher moistures (lower temperatures). Gomez et al (1988) reported that decorticated (abrasively dehulled) sorghum extruded at low moisture (18%) has a greater water solubility than sorghum extruded at higher moisture contents (32 and 45%). They surmised that water acts as a plasticizer, lubricating the feed material and reducing the friction and heat encountered during extrusion.

Changes in water solubility of starch caused by extrusion were studied by Mercier and Feillet (1975). Since no ethanol-soluble fraction was obtained from extruded starches, they concluded that extrusion increases the solubility of corn starch without formation of maltodextrins.

Subsequently, the apparent molecular weight (MW) profile of starch from extruded materials was characterized by low-pressure size-exclusion chromatography (Meuser et al 1982, Colonna and Mercier 1983, Colonna et al 1984) and by more rapid high performance liquid chromatography techniques (Kervinen et al 1985). Meuser et al (1982) showed chromatograms indicating a decrease in amylopectin MW after high-temperature (low-moisture) extrusion. Amylose apparent MW was little affected, and the overall starch MW range did not change. Colonna et al (1984) separated starch components in 0.1N KOH using gel-permeation chromatography. They reported a decrease in the amount of starch eluting in the column void volume after extrusion. Amylose and amylopectin (from extruded material and fractionated with selective precipitation with thymol and n-

butanol) had lower number average MWs and intrinsic viscosities than did raw starch, which indicated starch was partially depolymerized during extrusion.

Some uncertainty about the MW characteristics of extruded starch remain. Gomez and Aguilera (1983, 1984) presented a useful model suggesting that extrusion processes could be modeled by viewing the extrusion-damaged starch product as a combination of raw, gelatinized (2.5% starch cooked in water at 120°C for 1 hr), and dextrinized (gelatinized starch treated with α-amylase [EC 3.2.1.1]) starches. Starch extruded at low moistures closely resembled dextrinized starch in its water solubility and enzyme susceptibility properties, whereas starch extruded at high moistures more closely resembled gelatinized starch.

Characterization of water-soluble and water-dispersed components of extruded starch can lead to a better understanding of how extrusion affects the functional and chemical properties of extruded starchy foods. Previous workers have characterized the molecular changes in extruded starch by analyzing total starch and the proportion of amylose and amylopectin. Colonna et al (1989) stated that these techniques require exhaustive solubilization of starch samples using alkali or methyl sulfoxide; however, extruded materials are rarely exposed to these solvents in food systems. In processed foods, starch polymers exist in various forms: water-soluble individual molecules, water-soluble aggregates (microgels), and water-insoluble starch gel masses. Since these polymer forms would have different functional roles in food systems, it is important to develop methods that characterize these polymer forms in addition to total starch and the amylose/amylopectin ratio. The HPSEC method reported in this work is designed to characterize those polymers that are water-soluble as individual molecules ("molecularly soluble" polymers).

MATERIALS AND METHODS

Extruded corn starch (25% amylose) and extruded decorticated sorghum flours (12% amylose [waxy-normal blend], 22% amylose [heterowaxy, with one or two recessive waxy genes], and 27% amylose [normal or "nonwaxy"] with 84.7, 85.9, and 84.3% starch, respectively) were characterized by aqueous HPSEC. Extrusion conditions for the corn and sorghum were outlined by Gomez and Aguilera (1984) and Gomez et al (1988). The extrusion moisture content ranged from 11.3 to 32.2% for corn starch and from 17.5 to 44.8% for decorticated sorghum flours. Both the corn and sorghum extrudates were dried at low temperature (<55°C), ground and sieved (corn, 150 μm; sorghum, 250 μm). The ground samples were stored at 4°C prior to analysis. Conventional analysis of these extruded materials was presented earlier (Gomez and Aguilera 1983, 1984).

Corn starch and sorghum flour suspensions (1%) were prepared by bringing 1.0 g of sample and 0.5 ml of methanol to a volume of 100 ml with water (25°C). The small amount of methanol helped to "wet" the starch, assuring good dispersion. Samples (10.0 ml) of these suspensions were treated at different temperatures. Corn starch was solubilized in water for 10 min at 35°C. Extruded decorticated sorghum was solubilized for 10 min at 35, 65, and 85°C, or boiled for 10 min and then autoclaved for 10 min. All samples were sonicated for 20 sec after heat treatment. Extruded corn starch (1%) was also solubilized in 90% methyl sulfoxide for 15 min at 100°C but not sonicated. Each solution was centrifuged 10 min at 3,400 × g, and the supernatant was filtered through a 0.45-μm nylon filter.

Starch-containing solutions (25 μl) were injected into four S-series Shodex Ionpak columns (Showa Denko, K.K. Tokyo, Japan) connected in series (Jackson et al 1989). Water was used as the mobile phase. Carbohydrate polymers, separated on the basis of effective diameter, were detected using a differential refractive index detector (Waters model 410, Millipore Corp., Milford, MA). Starch peaks were identified using purified amylose and amylopectin from Sigma Chemical Co. (St. Louis, MO).

Starch MWs were estimated using pullulan (α-1,6 maltotriose) standards (Showa Denko K.K., Tokyo, Japan). Without direct MW measurements, pullulan standards serve as a basis for sample comparisons. Praznik (1987), using laser-light scattering methods, reported that pullulans have the same migration behavior in aqueous systems as debranched starch molecules, whereas dextrans do not. Pullulans, however, can only serve as MW estimators for amylopectin because their branching configurations differ and because the largest standard has an MW of 853,000 (Jackson et al 1988).

Sorghum extrudates were processed and analyzed in groups of similar amylose content, and the corn extrudates were processed and analyzed as one group. Each sample was replicated at least three times. HPSEC-determined water solubility was calculated by dividing the HPSEC-determined quantity of starch by the quantity of starch that would have been injected into the columns if the polymers were 100% soluble. Since starch polymers are not completely soluble as individual molecules in water, total HPSEC solubility does not reach 100%. Information on these techniques was presented earlier (Jackson et al 1989). Statistical analyses of solubility differences were conducted using SAS software release 6.03 (1987) for personal computers.

RESULTS AND DISCUSSION

Starch "Water Solubility"

Gelatinized or cooked starch polymers in solution can exist in several physical forms, i.e., trapped in granule remnants, entangled in gelled masses, as individual molecules, recrystallized (retrograded) polymers, and perhaps as combinations of the above forms. Processing conditions influence the distribution of these forms and thus the "water-solubility" of the sample.

Measures of starch water solubility also depend on the technique used to determine that property. The water-solubility index (a traditional non-HPSEC method), commonly used to characterize extruded samples, is a measure of the amount of starch (and other soluble components) that disperse after a sample is treated in 30°C water for 30 min and centrifuged; this "soluble" starch can be in the form of individual molecules of amylose and amylopectin, or as clumps (loose aggregates) of these molecules whose overall conformation, size, and degree of hydration allow them to stay dispersed after centrifugation.

The aqueous HPSEC procedure is not a measure of the total starch content of a sample; it is, however, a measure of starch polymers that are dispersed in water as nonaggregated amylose and amylopectin molecules, or products of their depolymerization. Before HPSEC analysis, starch dispersions are filtered through a 0.45-μm filter to assure that polymer aggregates are not analyzed. I'Anson et al (1985) found that the hydrodynamic spherical radius and radius of gyration of wheat amylopectin are 0.131 (diameter 0.262 μm) and 0.101 μm, respectively. Individual corn amylopectin

molecules were not filtered out by the 0.45-μm filter, since there were no differences in HPSEC chromatograms of identical solutions filtered with 5- or 0.45-μm filters.

Extruded Corn Starch

Representative chromatograms of extruded corn starches extracted at 35°C are presented in Figure 1. Based on pullulan standards, the apparent MW of amylopectin was 7.4×10^6 and the apparent MW of amylose was 1.9×10^5. All samples had similar apparent MW patterns to autoclaved and sonicated corn starch (Jackson et al 1989). Although the depolymerization of starch caused by other processes (twin-screw extrusion cooking, or limited enzymatic hydrolysis) was more evident on chromatograms produced by this aqueous HPSEC system (Jackson 1988), no formation of maltodextrins and no substantial quantities of soluble depolymerized fractions of amylose and amylopectin were observed. An increase in solubility between the amylopectin and amylose peaks is evident with decreasing extrusion moisture content; this increase represents partial amylopectin depolymerization. HPSEC of extruded starch, solubilized in 90% methyl sulfoxide, separated amylopectin and the depolymerized amylopectin fractions more clearly (Fig. 2). Starch polymers solubilized in methyl sulfoxide would not necessarily be those solubilized in a water-based food system. In an aqueous system these intermediately sized molecules appear less readily soluble, perhaps because they retrograde after extrusion.

Total corn starch solubility, as measured by HPSEC, increased with decreasing extrusion moisture content from 33 to 15% (Fig. 3). Interestingly, however, at extrusion moistures below 15%, starch solubility was lower. Gomez and Aguilera (1984) reported that the water solubility index of starches extruded below 15% increased dramatically, but the enzyme susceptibility did not change. This confirms that some starch products (retrograded or depolymerized) formed during or after very low moisture extrusion are less soluble in water as individual molecules. An increase in enzyme-susceptible starch would have occurred if individual molecules were solubilized, because, in general, greater polymer solubility increases enzymatic starch hydrolysis (Rollings et al 1983). Furthermore, when excessive sonication of autoclaved starch creates depolymerized amylopectin products with MWs $>10^5$, similar to fractions seen in Figure 2, starch solubility was reduced (Jackson 1988).

Workers who have shown that limited depolymerization occurs during extrusion have suggested that increased water solubility resulted from starch depolymerization (Meuser et al 1982, Colonna et al 1984) and/or an increased ability of depolymerized starch to leach from entangled molecules (Colonna and Mercier
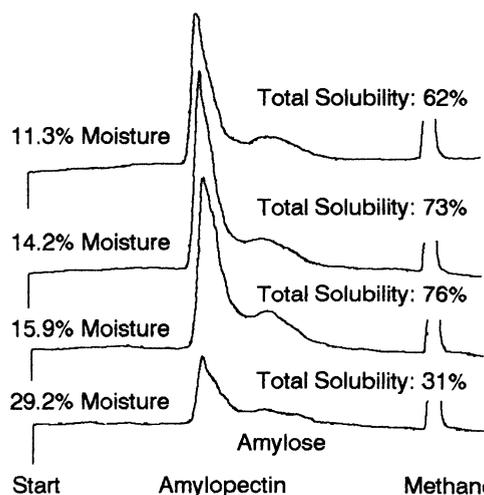

Fig. 1. Representative chromatograms of extruded corn starches extracted with water at 35°C. Starch solubiolity was calculated by dividing the quantity of starch detected by high-performance size-exclusion chromatography by the amount of starch that would have been injected into the columns if the starch were 100% soluble.

1983). Our aqueous HPSEC data indicate that at least part of the increased solubility observed by other workers is caused by an extrusion-induced increase in the molecular solubility and dispersibility of amylose and amylopectin. Hence, increased water solubility is not necessarily from the formation of depolymerized starch.

Extruded Decorticated Sorghum

Chromatograms of extruded sorghum flours showed separation of two amylopectin peaks (apparent MWs of 7.7×10^6 and 3.6×10^6, respectively) (Figs. 4 and 5). Representative chromatograms of nonwaxy sorghum extrudates are shown in Figure 4. Two amylopectin populations were also observed in pure (not extruded) sorghum starches (Jackson et al 1989). It is inappropriate to quantify the two amylopectin populations, however, because a clear delineation of polymer types under those curves is not possible. Increase in amylopectin solubility, however, is largely from the higher MW fraction (Fig. 4).

A small amount of oligosaccharides was soluble at 35°C (Figs. 4 and 5). These dextrins were not produced by extrusion, since they were also present in the uncooked sorghum flours. Starch solubility of sorghum extrudates extracted at 35°C generally increased with decreasing extrusion moisture content (Fig. 6). However, extrusion moisture contents were higher than those where corn starch solubility decreased (Figs. 1 and 3). The amount of soluble starch at 35°C was greater for all sorghum flours extruded at 32 and 18% moisture contents versus 45% moisture (Fig. 6).

Extruded nonwaxy (27% amylose) sorghum was also less soluble than the extruded heterowaxy (22% amylose) or the waxy-nonwaxy blend (12% amylose) (Fig. 5). Extruded nonwaxy sorghum starch, when extracted at temperatures below
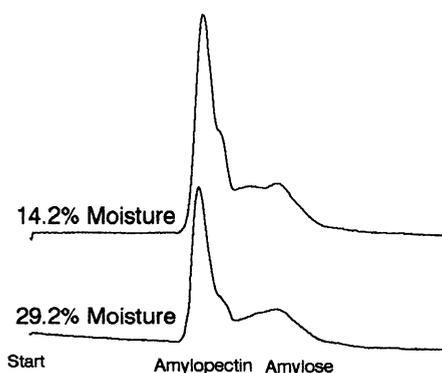
gelatinization, is less soluble than heterowaxy or waxy sorghum starches (Jackson et al 1989). Extrudates solubilized at 65 and 85°C (chromatograms not shown) had starch solubility trends similar to those exhibited in Figure 5. When granular structure is destroyed by extrusion, amylose is less soluble than amylopectin. Only when granules remain intact is amylose, on a percentage basis, more molecularly soluble than amylopectin (Jackson et al 1989). Therefore, extruded foods made with starches higher in amylopectin are likely to be more soluble in cold water than identical foods made with less amylopectin.

Autoclaved, extruded sorghum flour was more soluble than that solubilized at lower temperatures (Figs. 4 and 7). Combined treatments of extrusion and autoclaving confound the solubility differences between the sorghum samples, since autoclaving makes

Fig. 2. Chromatographic patterns of extruded corn starch extracted with methyl sulfoxide for 15 min at 100°C (no sonication).
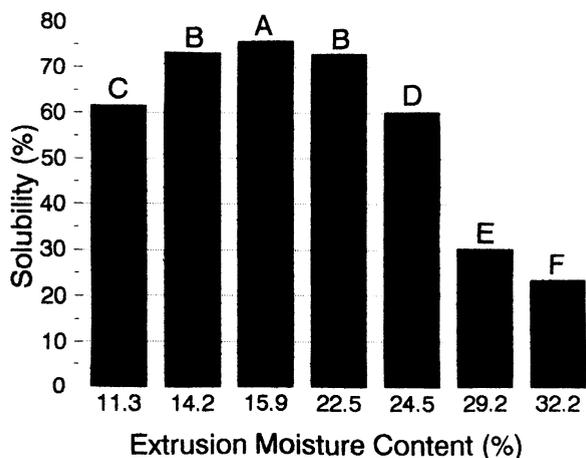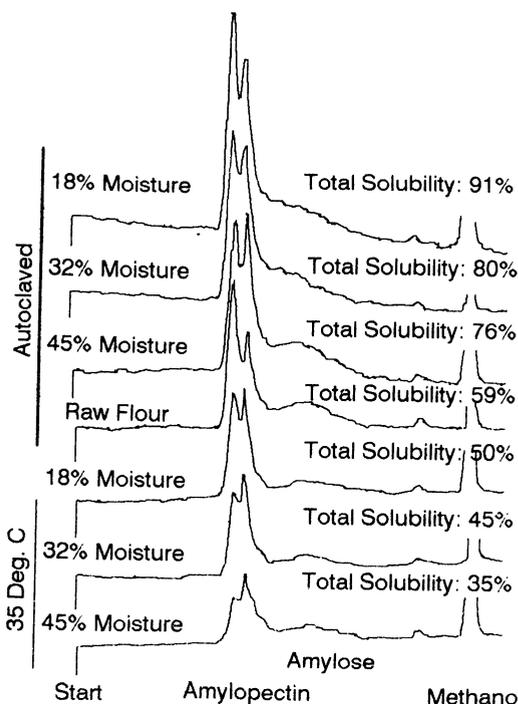
Fig. 3. Water solubility of extruded corn starch at 35°C. Starch solubility was calculated by dividing the quantity of starch detected by high-performance size-exclusion chromatography by the amount of starch that would have been injected into the columns if the starch were 100% soluble. Values with the same letter are not significantly different ($\alpha = 0.05$).

Fig. 4. Representative chromatograms of nonwaxy sorghum extracted with water at 35°C (bottom three chromatograms) or boiled and autoclaved in water (top four chromatograms). Starch solubility was calculated by dividing the quantity of starch detected by high-performance size-exclusion chromatography by the amount of starch that would have been injected into the columns if the starch were 100% soluble.
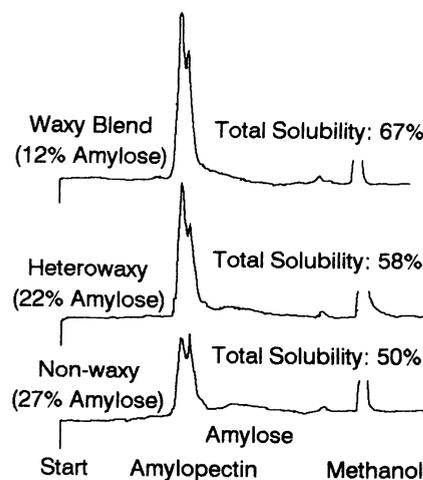
Fig. 5. Representative chromatograms of nonwaxy-waxy blend, heterowaxy, and nonwaxy sorghum extruded at 18% moisture and extracted with water at 35°C. Starch solubility was calculated by dividing the quantity of starch detected by high-performance size-exclusion chromatography by the amount of starch that would have been injected into the columns if the starch were 100% soluble.
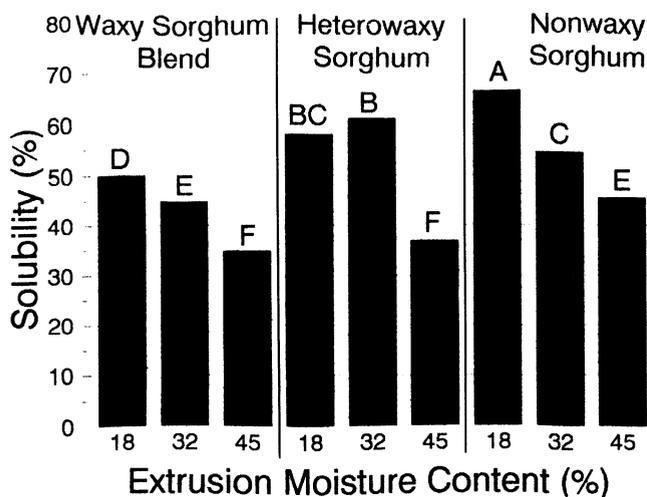
Fig. 6. Starch water solubility of extruded nonwaxy-waxy blend, heterowaxy, and nonwaxy decorticated sorghum flours at 35°C. Starch solubility was calculated by dividing the quantity of starch detected by high-performance size-exclusion chromatography by the amount of starch that would have been injected into the columns if the starch were 100% soluble. Values with the same letter are not significantly different ($\alpha = 0.05$).
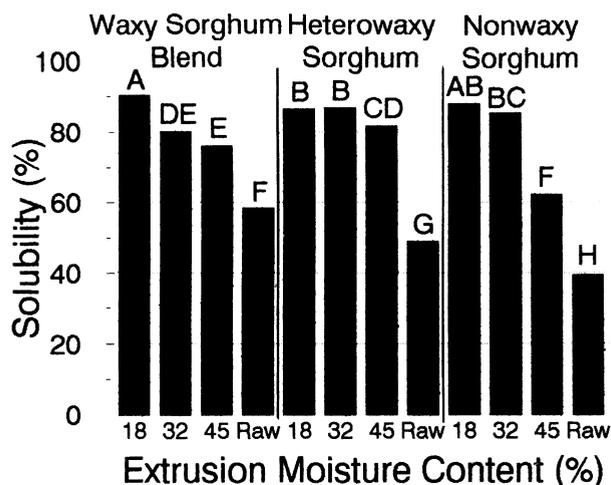
Fig. 7. Starch water solubility of extruded nonwaxy-waxy blend, heterowaxy, and nonwaxy (regular) decorticated sorghum flours after autoclaving. Starch solubility was calculated by dividing the quantity of starch detected by high-performance size-exclusion chromatography by the amount of starch that would have been injected into the columns if the starch were 100% soluble. Values with the same letter are not significantly different ($\alpha = 0.05$).

starch more soluble in the presence of excess water and extrusion does not. Starch solubilization at temperatures below gelatinization (35°C) are thus more valuable for HPSEC characterization of the inherent effects of extrusion.

CONCLUSIONS

Aqueous HPSEC can be successfully used to characterize starch from extruded cereals. Extruded foods containing high percentages of amylose are generally less water soluble than identically extruded foods with less amylose. Some differences in starch solubility were evident between nonextruded (raw flour) and extruded sorghum even after both materials were solubilized by autoclaving. This suggests that extrusion produced starch polymer and starch polymer aggregates that are different from those obtained after heating starch in excess water, and some of these extruded starch materials have an increased water solubility. However, under certain moisture and temperature conditions, starch polymer structures that had reduced water solubility were

created during and after corn starch extrusion. As with excessive sonication, these less water-soluble polymers could be those with reduced MW (but still within the MW range of amylose and amylopectin).

No maltodextrins were produced during these extrusion processes. Starch depolymerization has been reported to occur during extrusion processes, but these new polymers are not necessarily as molecularly soluble in water as nonextruded starch. HPSEC can be used to monitor the solubility changes of starch caused by extrusion or in other starch-modifying food process.

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