Quantitative Measurement of Extrusion-Induced Starch Fragmentation Products in Maize Flour Using Nonaqueous Automated Gel-Permeation Chromatography

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ABSTRACT

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Automated gel-permeation chromatography (GPC), with application of the universal calibration concept, was used to investigate the mechanism of extrusion-induced starch fragmentation in corn. High-amylose and high-amylopectin corn flours were subjected to twin-screw extrusion. The effects of moisture content, die temperature, screw speed, mass flow rate, and amylose-amylopectin ratio were investigated. Nonaqueous GPC, using a refractive index monitor and viscometer, yielded quantitative size profiles of native and extruded starches, as well as information describing branching patterns of the starch. Fragmentation was most pronounced in amylopectins with a molecular weight of 10^7–10^9, which yielded fragments of 10^4–10^7. Consistent with gravity-flow GPC with dimethyl sulfoxide, separation indicated that fragmentation was promoted at low temperatures. However, a correlation between degree of fragmentation and specific mechanical energy was not observed. Formulations containing high amylopectin levels were most prone to fragmentation. Die temperature significantly affected such textural properties as cohesiveness, springiness, gumminess, and chewiness of the extruded flours.

During extrusion, starch is subjected to high shear forces that physically cleave glycosidic linkages forming fragments of lower molecular weight. Wen et al (1990) used gravity-flow gel-permeation chromatography (GPC), with dimethyl sulfoxide (DMSO) as the mobile phase, to measure extrusion-induced starch fragmentation. The qualitative polysaccharide size distributions showed that the high molecular weight material decreased as moisture content and temperature were decreased and screw speed was raised.

Timpa (1991) developed a GPC procedure to determine the molecular weight distribution (MWD) of cellulose using dimethyl acetamide/lithium chloride (DMAC/LiCl) for solubilization and as the mobile phase. The technique uses viscosity and refractive index detectors, and enables data to be processed using the universal calibration concept (Grubisic et al 1967). MWD is based upon calibration with well-characterized narrow distribution polystyrene standards (Timpa 1991). Wasserman and Timpa (1991) used this technique to quantify extrusion-induced starch fragmentation in corn meal extrudates. They found that the largest molecules (molecular weight values of 10^7–10^8) were prone to fragmentation, yielding fragments in the weight range of 10^5–10^7.

Using a statistical design, the objective of this study was to apply nonaqueous GPC to make a comprehensive assessment of corn starch structural changes occurring as a result of twin-screw extrusion. Quantitative profiles of starch size distribution were obtained, and molecular size changes were correlated with extrusion operating conditions and textural properties. Results illustrate the applicability of nonaqueous GPC, with application of the universal calibration concept, to obtain rapid quantitative data showing processing-related modifications of polysaccharide structure.

MATERIALS AND METHODS

Materials

Corn meal was obtained from Lauhoff Grain Co., Danville IL. Analysis data provided by suppliers indicated the proximate composition: 12% moisture, 7% protein, 0.7% fat, 0.5% fiber, 0.4% ash, and 79.4% N-free extract. The two corn flours (National Starch, Bridgewater, NJ) evaluated in this study were a high-amylose flour (42% amylose and 18% amylopectin) and a high-amylopectin flour (60% amylopectin). Amylose and amylopectin from corn were obtained from Sigma Chemical Co., St. Louis, MO.

Extrusion

Extrusion was performed in a corotating twin-screw extruder (model ZSK-30, Werner & Pfleiderer). The extrusion conditions were selected based upon one-half fraction of a 2^5 factorial design (Table I), where the five factors were die temperature, screw speed, mass flow rate, moisture content, and amylose-amylopectin ratio. Due to low moisture conditions and limitations of the extruder, there were three points in the design that could not be processed. Extruder specifications and screw configuration were: barrel bore diameter, 30.9 mm; screw length, 878 mm; maximum screw diameter 30.7 mm; kneading blocks at 440 mm (45/5/14), 480 mm (45/5/14), 538 mm (45/5/20), 592 mm (45/5/28), and 620 mm (45/5/14 LH); igels at 210 mm (42/42), 336 mm (42/42); die opening, 3.0 mm.

TABLE I
Factorial Design for Corn Flour

Material Sample	% Moisture (w/w)	Total Mass Flow Rate (g/min)	Screw Speed (rpm)	Die Temperature (°C)
High Amylopectin				
G33	20	400	500	180
G31	20	400	250	140
G32	20	200	500	140
G34	20	200	250	180
NA[a]	12.5	400	500	140
G36	12.5	400	250	180
G35	12.5	200	500	180
NA	12.5	200	250	140
High Amylose				
G25	20	400	500	140
G27	20	400	250	180
G28	20	200	500	180
G26	20	200	250	140
G24	12.5	400	500	180
NA	12.5	400	250	140
G30	12.5	200	500	140
G29	12.5	200	250	180

[a] Not available due to limitations of the extruder.

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Specific mechanical energy (SME) was calculated for each extruded sample. SME (Whr/kg) is defined as the product of the torque (N × m) times the rotational screw speed (sec^{-1}) divided by the mass flow rate (kg/hr) (van Lengrich 1990).

GPC

The methodology of Timpa (1991) was applied to the corn samples, with procedures for sample preparation, GPC, data acquisition, and analysis as described in Politz et al (1994).

Mark-Houwink plots, showing the relationship between intrinsic viscosity and molecular weight (Yau et al 1979), were obtained for the corn amylose and amylopectin standards for comparison with the unprocessed flours.

Texture Profile Measurements

Texture profile analysis (TPA) parameters (hardness, cohesiveness, springiness, gumminess, and chewiness) were determined according to the methods of Halek et al (1989) and Bourne and Comstock (1981). Measurements were taken with an Instron universal testing machine (model TM, Instron Corp., Canton, MA) as described in Politz et al (1994).

Differential Scanning Calorimetry and Polarized Light Microscopy

Programs were obtained using a DSC-7 Perkin Elmer differential scanning calorimeter equipped with an intercooler (Politz et al 1994).

Statistical Analysis

Statistical results were obtained using the SAS program (SAS 1989). Parameters used for the analysis of variance (ANOVA) included extrusion conditions, MWD, and TPA values. Simple linear regression was conducted to study the relationship between SME and the MWD. Correlation analysis was performed on the MWD and TPA values.

RESULTS AND DISCUSSION

Extrusion-Induced Starch Fragmentation

Corn meal and two varieties of corn flour were extruded under 13 different operating conditions (Table I). Operating variables studied included moisture content, screw speed, die temperature, mass flow rate, and amylose-amylopectin ratio.

GPC profiles of native corn meal were similar to those of previous analyses (Wasserman and Timpa 1991). MWD of native and extruded starches is illustrated in Figures 1 and 2. Each chromatogram provides a graphic representation of weight fraction versus logarithm of molecular weight (Yau et al 1979). Starch size in unprocessed flours ranged from 18.8 × 10^6 to 2.3 × 10^6. These values are 5–10% higher than previously conducted characterizations of corn starch using aqueous GPC with refractive index and light-scattering detectors (Takeda et al 1988). All samples were solubilized in DMAC/LiCl for comparison of peak areas.

After extrusion, extensive fragmentation, characterized by downshifts in MWD, was observed in all samples (Figs. 1 and 2). Both MWD and cumulative molecular weight plots (Fig. 3) show that the largest starch molecules (in the range of 10^7 to 10^9) were most prone to fragmentation. The loss of high molecular weight (HMW) starch was accompanied by the production of fragments in the weight range of 10^4–10^8. Data taken from the cumulative

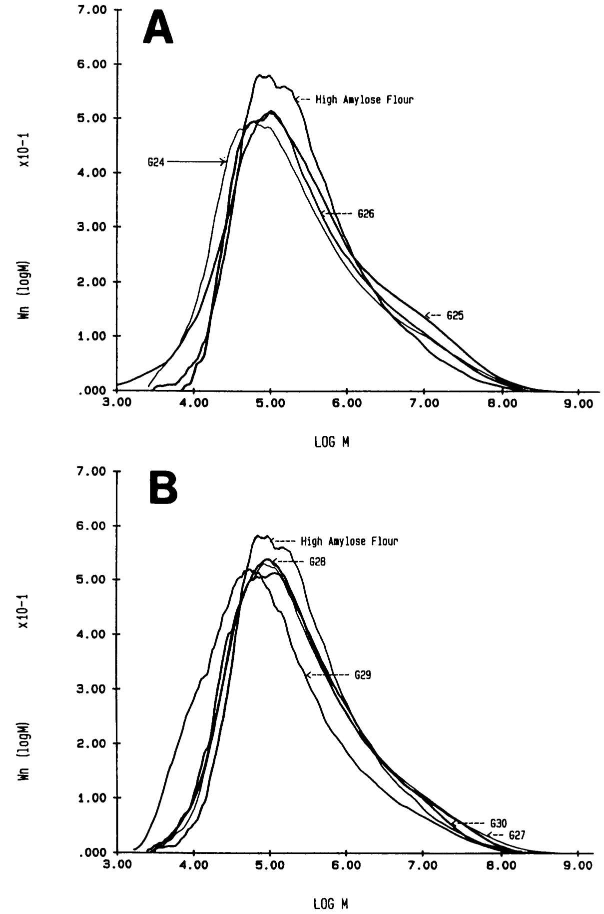

Fig. 2. Molecular weight distribution profiles of native high-amylose corn flour and extrudates. **A,** Three extrudates. **B,** Four extrudates. Processing conditions are defined in Table I.

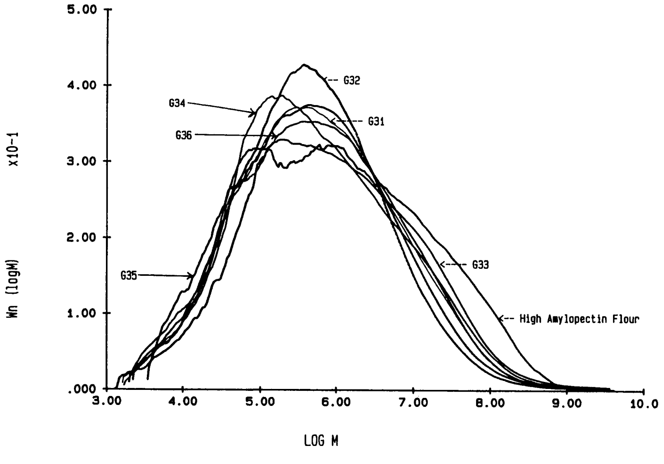

Fig. 1. Molecular weight distribution profiles of native high-amylopectin corn flour and extrudates. Processing conditions are defined in Table I.

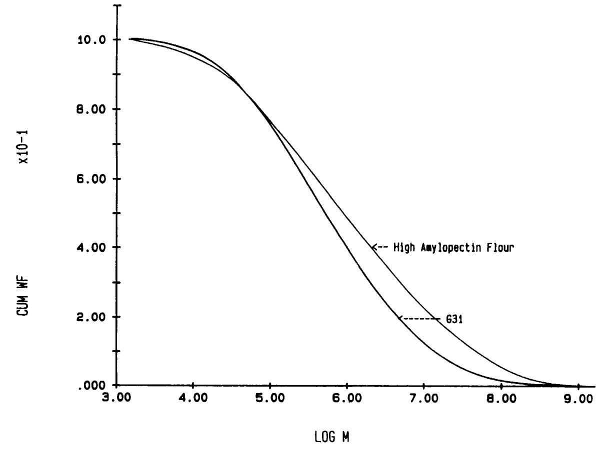

Fig. 3. Cumulative molecular weight plots for native high-amylopectin corn flour and extrudate G31.

MWD plots (Fig. 3) were used to determine the relative abundance of molecules falling within specific molecular size ranges (Tables II and III). There was a reduction (8–17%) in fragments of molecular weight $>10^7$ for the high-amylopectin samples, with a corresponding increase in fragments of 10^6–10^5. Similarly, high-amylose extrudates exhibited an apparent reduction of fragments in the molecular weight range of 10^6–10^5, with a corresponding increase in fragments of 10^5–10^4.

ANOVA pinpointed specific variables responsible for promotion of starch fragmentation, based upon weight average molecular weight (M_w) and number average molecular weight (M_n) values (Table IV). ANOVA showed that the amylose-amylopectin ratio was the primary factor significantly affecting fragmentation of the high-amylose and high-amylopectin corn flours evaluated in this study. Significant decreases in M_w (from 18.8×10^6 to 5.6×10^6) were observed only for the high-amylopectin extrudates ($P = 0.0001$). Similarly, declines in M_n also occurred (Table IV) ($P = 0.0542$). In agreement with previous results using conventional GPC, Colonna et al (1984) estimated that fragmentation of the amylopectin following extrusion is 10 times greater than it is for amylose. In addition, both amylose and amylopectin (from extruded material and fractionated by selective precipitation with thymol and *n*-butanol) had lower M_n values and intrinsic viscosities than did the raw starch, which indicated the starch was fragmented during extrusion (Colonna et al 1984).

The high-amylopectin flour appears to fragment under low temperature conditions at 20% moisture. Due to limitations of the extruder, it was not possible to extrude samples at lower moisture levels and temperatures for comparison. At 12.5% moisture, high screw speed appeared to promote fragmentation more than low screw speed did. These findings are consistent with previous studies (Davidson et al 1984, Chinnaswamy and Hanna 1990, Wen et al 1990). SME did not significantly correlate with the molecular size results.

Branching Results

It is well established that polymers possessing identical hydrodynamic volumes ($\eta \times$ molecular weight) coelute from a chromatography column. Moreover, branches on a polymer chain in solution reduce the hydrodynamic volume of the polymer molecule relative to a linear molecule of the same molecular weight (Yau and Rollings 1987). Theoretical principles for using GPC to characterize branching patterns of polysaccharides were explained by Yau and Rollings (1987). An approximation of the degree of branching in the unprocessed and extruded samples was obtained with pure amylose and amylopectin serving as controls. The parameter λ serves as a qualitative index of average polymer branching frequency. A reduction in average λ values (Table IV) for the extrudates relative to the unprocessed controls was observed (e.g., reduction from 0.12 to 0.02–0.09 for the high-amylopectin flour). These results indicate that fragmentation occurred mainly in the amylopectin component of the starch. As a qualitative confirmation, average λ values were obtained for pure corn amylopectin and amylose (Table V). The value obtained for corn amylopectin (0.13) is close to the result for

TABLE II
Cumulative Molecular Weight Distributions (MWD) (%) of High-Amylopectin Corn Flour Before and After Extrusion[a]

Sample	$\geq 10^7$	10^7–10^6	10^6–10^5	10^5–10^4
Control	26	26	27	18
G31	13	27	37	19
G32	8	29	39	21
G33	17	26	32	21
G34	14	26	36	21
G35	12	34	37	15
G36	13	29	34	20

[a] Data taken from the cumulative MWD plots (Fig. 3) were used to determine the relative abundance of molecules falling within specific molecular weight ranges.

TABLE III
Cumulative Molecular Weight Distributions (MWD) (%) of High-Amylose Corn Flour Before and After Extrusion[a]

Sample	$\geq 10^7$	10^7–10^6	10^6–10^5	10^5–10^4
Control	6	19	45	29
G24	6	14	37	39
G25	7	17	41	33
G26	6	18	38	33
G27	6	15	40	36
G28	7	18	42	31
G29	6	13	36	38
G30	6	16	41	35

[a] Data taken from the cumulative MWD plots (Fig. 3) were used to determine the relative abundance of molecules falling within specific molecular weight ranges.

TABLE IV
Specific Mechanical Energy (SME) and Molecular Weight Results for High-Amylopectin and High-Amylose Corn Flours Before and After Extrusion[a]

Sample	SME (kJ/kg)	M_w[b] ($\times 10^6$)	M_n[c] ($\times 10^4$)	M_w/M_n[d]	Average λ[e]
High-amylopectin control		18.8	11.9	158	0.12
G31	519	7.4	8.3	89	0.02
G32	1,386	6.0	8.0	75	0.03
G33	827	12.0	7.3	164	0.04
G34	452	10.7	9.4	114	0.09
G35	1,358	5.6	7.8	72	0.09
G36	553	8.9	7.0	127	0.02
High-amylose control		2.3	14.1	16	0.02
G24	680	2.4	7.4	32	0.006
G25	531	2.7	10.7	25	0.002
G26	525	2.6	5.1	51	0.002
G27	303	1.6	7.5	21	0.004
G28	630	2.9	9.4	31	0.001
G29	863	2.1	4.0	53	0.001
G30	1,709	1.7	9.5	18	0.003
P-value[f]		0.0001	0.0542	0.003	

[a] Samples were dissolved and chromatographed as described in Materials and Methods.

[b] Weight average molecular weight.

[c] Number average molecular weight.

[d] Polydispersity ratio.

[e] Qualitative index of average polymer branching frequency.

[f] From the test for differences in means between high-amylopectin and high-amylose flours.

native high-amylopectin flour (0.12). The value obtained for the high-amylose flour is 0.02, which is well below the value for amylopectin, but higher than pure amylose. The presence of protein and other components of the flour could account for this difference.

Mark-Houwink Plots

Mark-Houwink plots of the unprocessed flours and corn amylose and amylopectin are shown in Figure 4. The curve for the high-amylose flour parallels that of pure amylose, demonstrating the relationship between intrinsic viscosity and molecular weight in the samples used in this study. The slope of the high-amylopectin flour curve is slightly lower than that of pure amylopectin, which could be due to the interaction between the starch and protein in the flour or the different botanical starch source.

Gelatinization Characteristics

DSC was conducted using native and extruded flour samples containing 70% water. In unprocessed samples, which were scanned over a temperature range of 20–120°C, a gelatinization peak (data not shown) was observed in accordance with established values (Whistler and Daniel 1985). After extrusion, these peaks disappeared, which clearly indicates total loss of ungelatinized starch. These results are in accordance with Chinnaswamy et al (1989) and Gomez and Aquilera (1984). Each of the samples retained a melt peak (Donovan 1979, Wang et al 1989) between 200 and 255°C when no added water was present during DSC. Polarized light microscopy showed loss of birefringence in all extrudates examined.

Textural Properties

TPA values obtained for the extrudates are shown in Table VI. ANOVA of extrusion conditions with TPA parameters showed that low temperature (140°C) results in extrudates with high cohesiveness, springiness, gumminess, and chewiness values versus samples processed at high temperature (180°C). In addition, high-amylopectin extrudates had significantly lower springiness values when compared to the high-amylose extrudates ($P = 0.0405$).

TABLE V
Molecular Weight Results for Pure Corn Amylose and Amylopectin[a]

Sample	M_w[b] ($\times 10^6$)	M_n[c] ($\times 10^6$)	M_w/M_n[d]	Average λ[e]
Amylopectin	26.5	10.1	1.5	0.13
Amylose	0.46	0.25	1.8	0.001

[a] Samples were dissolved and chromatographed as described in Materials and Methods.

[b] Weight average molecular weight.

[c] Number average molecular weight.

[d] Polydispersity ratio.

[e] Qualitative index of average polymer branching frequency.

Correlation analysis was used to relate molecular size profiles with TPA values. Changes in M_w (Table IV) correlated with springiness ($r = -0.60$), but the other TPA parameters evaluated did not. Whereas the amylose-amylopectin ratio significantly affected starch size, strict correlations between the degree of starch fragmentation and textural properties were not observed.

Compared to previous research using gravity-flow separations in DMSO (Wen et al 1990, Rodis et al 1993), GPC in DMAC/LiCl provides rapid and quantitative molecular size profiles of native and processed starches. Automated GPC yields important information that includes MWD, M_w, and M_n values, as well as cumulative molecular weight profiles. However, further research is necessary to quantitatively define relationships linking extruder operating parameters, starch structure, and textural properties.

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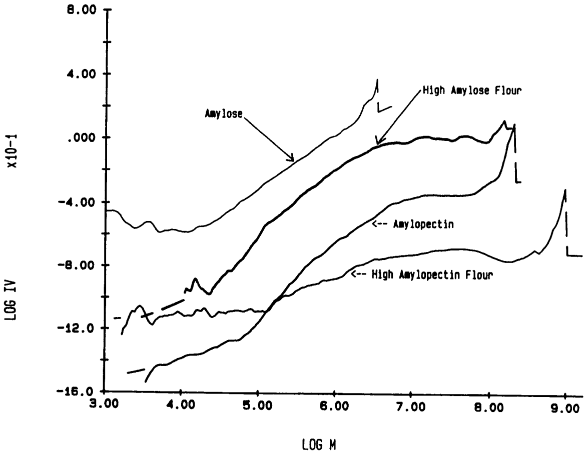

Fig. 4. Mark-Houwink plots of native high-amylose and high-amylopectin corn flours and pure corn amylose and amylopectin.

TABLE VI
Texture Profile Analysis Parameters of High-Amylose and High-Amylopectin Corn Flour Extrudates[a]

Extrudate	Hardness (MPa)	Cohesiveness	Springiness (mn)	Gumminess (MPa)	Chewiness (MPa·m)
High-amylose					
G24	0.10	47.4	8.8	4.9	43.2
G25	0.92	26.4	9.2	21.2	194.9
G26	3.17	18.7	8.9	53.0	475.9
G27	0.17	17.2	8.1	3.1	24.9
G28	0.30	56.1	8.5	15.8	135.3
G29	0.30	23.9	8.7	7.3	63.7
G30	0.10	137.5	8.9	14.6	129.6
High-amylopectin					
G31	0.60	220.6	8.9	52.9	472.7
G32	0.27	48.4	9.1	11.8	106.5
G33	0.68	14.9	7.1	10.3	72.7
G34	0.60	11.5	7.4	6.9	51.1
G35	0.54	5.9	6.3	2.9	18.4
G36	0.10	17.5	7.8	1.7	13.4
P-value[b]	0.160	0.056	0.015	0.008	0.006

[a] Obtained according to the method of Halek et al (1989) and Bourne and Comstock (1981).

[b] From the test for differences in means between high-amylopectin and high-amylose flours.

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