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Non-aqueous gel permeation chromatography of wheat starch in dimethylacetamide (DMAC) and LiCl: extrusion-induced fragmentation

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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 wheat. High and low protein flours were subjected to twin-screw extrusion and the effects of moisture, die temperature, screw speed, mass flow rate and protein content on starch structure and textural properties were investigated by non-aqueous GPC in dimethylacetamide (DMAC) and LiCl. This solvent system allowed for complete dissolution of the native and extruded starches. The use of refractive index and viscosity detectors enabled application of Mark–Houwink calculations to obtain quantitative size profiles of unprocessed and processed starch, and information describing branching patterns of the starch. Fragmentation was most pronounced in amylopectins of MW 10^7–10^8, which yielded fragments of MW 10^5–10^7. Of the operating parameters investigated, low die temperature and low moisture content led to extensive fragmentation. Methylation analysis showed only modest changes in linkage distributions, with little or no increase in terminal glucose indicating few fragmentation points relative to the total number of glycosidic linkages present. The lack of dextrins or oligosaccharides suggests that fragmentation occurs primarily in the B chains of amylopectin. Interactions between die temperature and moisture content were shown to significantly effect the hardness, cohesiveness, springiness, gumminess and chewiness of the extruded flours.

INTRODUCTION

Amylopectin undergoes various degrees of fragmentation during extrusion cooking depending upon operating parameters and the types of starch utilized (Colonna & Mercier, 1983; Colonna *et al.*, 1984; Davidson *et al.*, 1984; Chinnaswamy & Hanna, 1990; Wen *et al.*, 1990; Yamada *et al.*, 1990; Rodis *et al.*, 1993). The extent to which fragmentation occurs is thought to influence the rheological properties of the extrudates. Relationships between extrusion parameters and starch fragmentation in corn has recently been described (Wen *et al.*, 1990; Rodis *et al.*, 1993). However, data describing the mechanisms of fragmentation in wheat, which contains a higher level of protein than corn, are limited. Furthermore, most previous studies on starch size have employed classical gel filtration or permeation techniques, yielding data of a non-quantitative nature.

More recently, molecular weight determinations of polysaccharides such as cellulose (Timpa, 1991; Timpa & Triplett, 1993) and starch (Wasserman & Timpa, 1991) have been conducted using gel permeation chromatography (GPC), i.e. size exclusion chromatography (SEC), with combined detection by refractometry and viscometry employing dimethylacetamide/lithium chloride (DMAC/LiCl) for solubilization and as the mobile phase. Incorporation of dual viscometry and refractive index (RI) detectors enables application of the universal calibration concept (Grubisic *et al.*, 1967) to obtain molecular weight distributions (MWDs) based on calibration with well-characterized, narrow distribution, polystyrene standards (Timpa, 1991).

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Here, we utilize the combined approach of automated GPC and methylation analysis to characterize changes of starch structure in wheat flours occurring as the result of twin-screw extrusion. The specific objectives of this study were to obtain quantitative profiles of starch size distributions and to correlate molecular size changes with extrusion operating conditions and textural properties. Moreover, methylation analysis was used to determine bulk changes in glycosidic linkage distribution. Our results show fragmentation occurs to a greater extent in wheat, relative to corn (Wen *et al.*, 1990; Rodis *et al.*, 1993), but these changes are due to the cleavage of only a small fraction of glycosidic linkages.

EXPERIMENTAL

Materials

Two hard wheat flours ('Bouncer' and 'Boss', Bay State Milling Co., Winona, MN) were evaluated in this study. Analysis provided by the supplier lists 'Boss' flour with a proximate composition (w/w) of 73% starch (18% amylose and 55% amylopectin), 11·4% protein, 12–14% moisture, 0·5% ash and $<1\%$ lipid. 'Bouncer' flour contains a slightly higher level (14%) of protein. DMAC dried with molecular sieves (Type 4A, Baker) was obtained from Burdick & Jackson. LiCl (oven dried and stored desiccated) was from Baker. Polystyrene standards with nominal molecular weights of $1{\cdot}26 \times 10^6$, $4{\cdot}39 \times 10^4$, $3{\cdot}55 \times 10^5$ and $1{\cdot}96 \times 10^4$ were purchased from Toyo Soda Manufacturing (Tokyo, Japan). DMSO, *n*-butyllithium, and methyl iodide were purchased from Sigma Chemical Co. (St Louis, MO).

Extrusion

Extrusion was performed in a Werner & Pfleiderer co-rotating twin screw extruder (Model ZSK-30). Extrusion conditions were selected based on a one half fraction of a 2^5 factorial design (Table 1) where the five factors were die temperature, mass flow rate, throughput and moisture and protein levels. Extruder specifications and screw configuration were as follows: barrel bore diameter, 30·9 mm; screw length, 878 mm; 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.

GPC

The methodology of Timpa (1991) adapted to wheat flour as follows was used. Starch extrudates were ground in a Wiley Mill to pass a 40 mesh screen. Flours (45 mg) were added to 5 ml of DMAC in 10 ml Reacta-Vials (Pierce, Rockford, IL) in a heating block. The temperature was raised to 150°C for 1·25 h. After cooling to 100°C, dried LiCl (to 8% w/v) was added. The vials were capped, agitated by hand and heated at 100°C for 1 h. The temperature was then lowered to 50°C, and the vials were stirred overnight. The next day, vials were removed and placed on a reciprocating shaker for 30 min. The samples were then returned to the heating block and incubations continued (50°C) until the samples were visually clear. Then, solutions were quantitatively diluted to 50 ml with DMAC. Prior to injection, solutions were filtered *in vacuo* through Teflon solvent-resistant disposable filters (Millex SR, 0·5 μm, Millipore) using 4 ml glass vials (WISP, Waters) in a Baker 10 extraction apparatus fitted with glass syringes (10 cm^3).

Table 1. Factorial design for wheat flour

Sample	Protein (%)	Moisture (%) (w/w)	Total mass flow rate (g/min)	Screw speed (rev/min)	Die temp. (°C)
G45	14·0	20	400	500	185
G50	14·0	20	400	300	160
G51	14·0	20	225	500	160
G46	14·0	20	225	300	185
G40	14·0	16	400	500	160
G43	14·0	16	400	300	185
G44	14·0	16	225	500	185
G39	14·0	16	225	300	160
G47	11·4	20	400	500	160
G49	11·4	20	400	300	185
G48	11·4	20	225	500	185
G52	11·4	20	225	300	160
G42	11·4	16	400	500	185
G37	11·4	16	400	300	160
G38	11·4	16	225	500	160
G41	11·4	16	225	300	185

The mobile-phase solvent for GPC was DMAC containing 0·5% LiCl. This solution was prepared by adding dried LiCl to 1 liter of DMAC at 100°C, followed by filtration through a Teflon filter (Type FH, 0·5 μm, Millipore). The GPC system consisted of an automatic sampler (Waters WISP) with an HPLC pump (Waters Model 590), pulse dampener (Viscotek), viscometer detector (Viscotek Model 100), and refractive index detector (Waters Model 410) connected in series. This system was equipped with four columns (Ultrastyragel 10^3, 10^4 and 10^5 (Baxter, Muskegon, MI) and 10^6 (Phenomenex, Torrance, CA)) connected in series and preceded by a guard column (Phenogel, linear, Phenomenex). The system was maintained at 80°C.

Standard injection volumes were 400 μl and the mobile phase was pumped at a rate of 1·0 ml min^{-1}. Run times were 60 min. The software package Unical based upon ASYST (Unical, Version 3.02, Viscotek) was used for data acquisition and analysis. The system was calibrated with the polystyrene standards. Data were obtained from two dissolutions per sample with two GPC runs per dissolution.

This system provided plots from each detector which were then used for molecular weight determination. The calibration curves plotted logarithm of the product of intrinsic viscosity and molecular weight versus retention volume. MWD plots were obtained by dividing the partially integrated areas by the total area (100%) under the curve (Yau *et al.*, 1979).

The parameter λ, which serves as a qualitative index of average polymer branching frequency, was calculated based upon the equations of Styring *et al.* (1987) and Vilenchik and Ayotte (1992) using the Unical 3.02 software package.

Methylation analysis

Samples (300 mg) were defatted and starch was extracted in DMSO as described (Rodis *et al.*, 1993), with centrifugations at $10\,000\times g$ for 15 min and $20\,000\times g$ for 10 min. Samples were derivatized for glycosidic linkage analysis by methylation with *n*-butyllithium (York *et al.*, 1985; Carpita & Shea, 1989). Reaction products, dried over phosphorus pentoxide, were dissolved in 0·2 to 0·5 ml DMSO and *n*-butyllithium was added slowly, followed by methyl iodide. Methylated samples were extracted with chloroform, washed with water, and the $CHCl_3$ phase was evaporated to dryness. Samples were fully hydrolysed with 2 M trifluoroacetic acid, 121°C, 1 h, and reduced with $NaBD_4$. Per-*o*-methylated alditols were acetylated with acetic anhydride and 1-methylimidazole as a catalyst (Blakeney *et al.*, 1983). Gas chromatography–mass spectrometry (Hewlett-Packard model 5890 GC, 5970 MS, with HP-UX Series 300 Chem Station) was performed using a 30 m SP-2330 fused silica capillary column (Supelco Inc., Bellefonte, PA) and an oven temperature program with an initial temperature of 170°C for 3 min followed by a temperature ramp of 4 °C min^{-1} up to 240°C for 10 min. Injection was splitless for 0·75 min and split thereafter. Glycosyl-linkage analysis results are expressed as mol% (Sweet *et al.*, 1975).

Texture profile measurements

Texture profile analysis (TPA) parameters (hardness, cohesiveness, springiness, gumminess, and chewiness) were determined according to the method of Halek *et al.* (1989) and Bourne and Comstock (1981). Measurements were made with an Instron Universal Testing Machine, Model TM (Instron Corporation, Canton, MA). Wheat flour extrudates were subjected to two successive compressions (bites), which were performed on 10 mm specimens until each sample was compressed to 75% of its initial length (75% strain). The deformation speed of the cross-head was 25 mm min^{-1}. Four diameter measurements were taken on each specimen with a caliper before the first compression and were used to calculate the initial cross-sectional area. Averaged values are based upon 20 replicates for each sample.

Differential scanning calorimetry and polarized light microscopy

Thermograms were obtained using a DSC-7 Perkin Elmer differential scanning calorimeter equipped with an intercooler. Hermetically sealed stainless steel pans were used to prevent water loss. Samples (10–20 mg) were analyzed at a scanning rate of 5°C min^{-1}. A scan range of 20–120°C was used for samples containing 70% water and a scan range of 40–235°C used for samples without added water. Native flour and starch extrudates were examined with an Olympus BH-2 polarized light microscope at 100× using a crossed polar and a half wave filter to determine if any ungelatinized starch was still present after extrusion.

Statistical analysis

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

RESULTS AND DISCUSSION

Characteristics of the GPC system

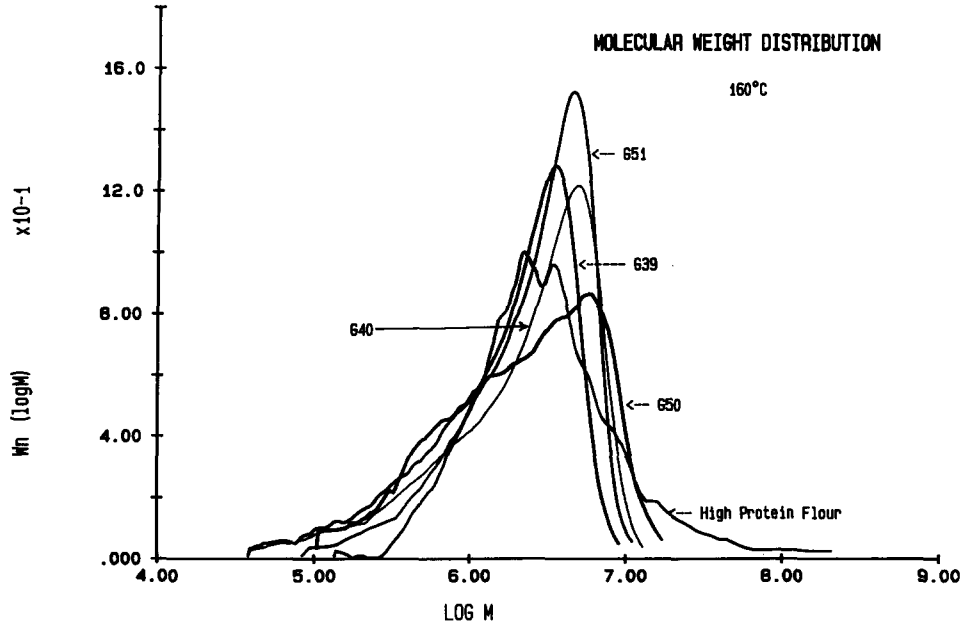
In a pilot study (Wasserman & Timpa, 1991) which investigated extrusion-induced fragmentation of corn starch, it was demonstrated that automated GPC utilizing DMAC and LiCl for dissolution and as a mobile phase, yields quantitative molecular weight distributions of starch. Relative to cellulose, for which this system was originally developed, starch readily dissolves in DMAC/LiCl. The salt (LiCl) is an important component of the mobile phase since it helps to maintain the solubility of large polysaccharides (Timpa, 1991). DMAC/LiCl produces homogeneous solutions of polysaccharides with negligible degradation (Dawsey & McCormick, 1990; Timpa, 1991).

GPC profiles of native and extruded wheat starches are illustrated in Figs 1 and 2. Each chromatogram provides a graphical representation of weight fraction versus the logarithm of MW (Yau *et al.*, 1979). All samples were solubilized in DMAC/LiCl based on a comparison of the areas under the MWD profiles. The system also provides weight average (MWw) and

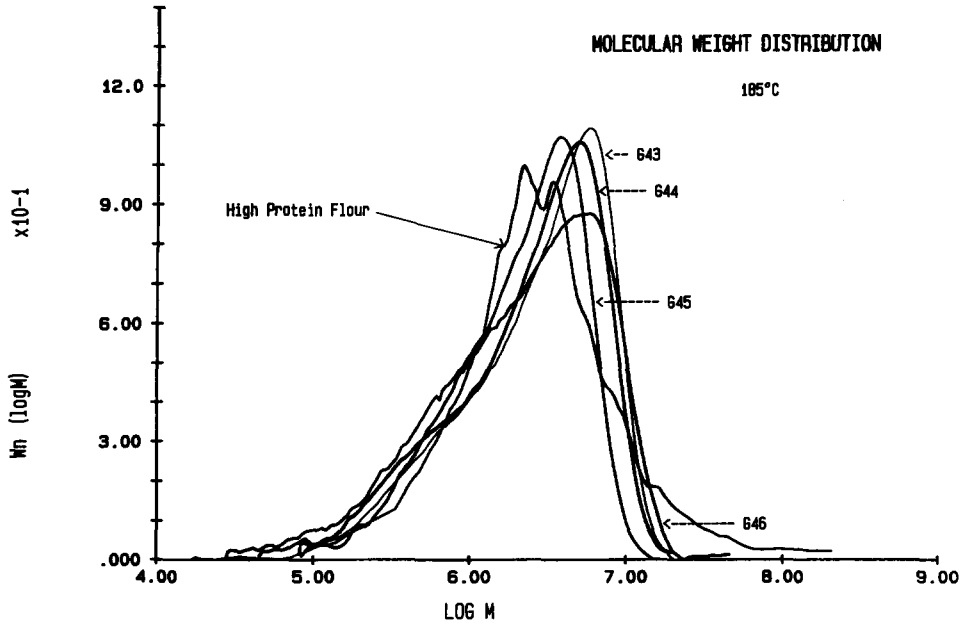
number average (MWn) molecular weight values (Tables 2 and 3). Furthermore, cumulative molecular weight plots (Fig. 3) can be generated. The cumulative molecular weight plots provide a convenient means for estimating specific populations over ranges of MW values (Timpa & Triplett, 1993). Starch size in unprocessed flours generally ranged between $5{\cdot}3 \times 10^6$ and $6{\cdot}5 \times 10^6$. These values fall within the range of previously conducted characterizations of wheat starch (Colonna *et al.*, 1984; Hizukuri & Maehara, 1990).

Extrusion-induced starch fragmentation

The two varieties of wheat flour were extruded under sixteen different extrusion conditions (Table 1). Operating variables studied included moisture content, screw speed, die temperature, mass flow rate, and protein content. Extensive fragmentation, characterized by a downshift of molecular weight profiles was observed in all samples (Figs 1 and 2). As seen in both MWDs (Figs 1 and 2) and in cumulative MW plots (Fig. 3), the



(a)



(b)

Fig. 1. Molecular weight distribution profiles of native high protein wheat flour and extrudates: (A) processed at 160°C; (B) processed at 185°C. Processing conditions are defined in Table 1. Wn (log MW) represents the weight fraction of material detected corresponding to each molecular weight value.

largest starch molecules (in the MW range of 10^7–10^8) were most prone to fragmentation. Data taken from the cumulative MWD plots (e.g. Fig. 3) were used to determine the relative abundance of molecules falling within specific molecular size ranges (Tables 4 and 5). For example, starch greater than MW of 10^7 was no longer detected following extrusion. The loss of this high molecular weight starch was accompanied by the production of fragments in the weight range of 10^5–10^7. In 11 out of the 16 extrudates, a significant reduction in fragments between 10^7 and 10^6 was also apparent. The magnitude of this downshift in wheat is more pronounced than seen in corn (Wasserman & Timpa, 1991; Politz *et al.*, 1994), and is consistent with earlier observations based upon gravity-flow GPC of wheat starch in KOH (Colonna *et al.*, 1984).

To determine which specific variables promoted starch fragmentation, ANOVA based upon the MWw

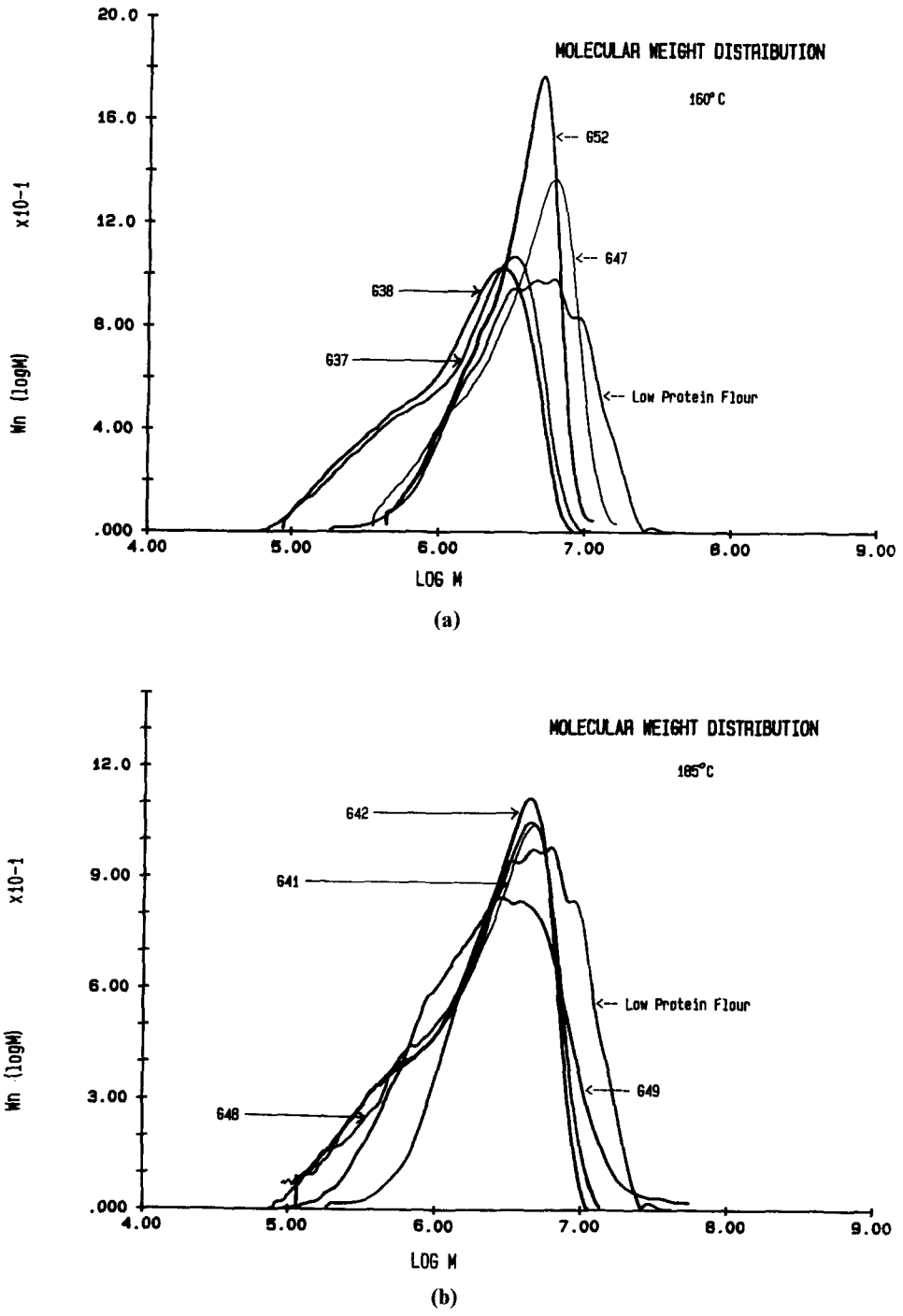


Fig. 2. Molecular weight distribution profiles of native low protein wheat flour and extrudates: (A) processed at 160°C; (B) processed at 185°C. Processing conditions are defined in Table 1. Wn (log MW) represents the weight fraction of material detected corresponding to each molecular weight value.

Table 2. Specific mechanical energy and molecular weight results for high protein (14%) wheat flour before and after extrusion[a]

Sample	SME (kJ/kg)	Weight avg. mol. wt (MWw) $\times 10^6$	Number avg. mol. wt (MWn) $\times 10^6$	Poly-dispersity ratio (MWw/MWn)	Average lambda
Control	—	6·53	2·20	3·0	0·021
G39	533	2·47	0·51	4·8	0·002
G40	518	3·27	0·85	3·8	0·000
G43	356	4·10	1·01	4·1	0·001
G44	656	3·79	1·02	3·7	0·001
G45	356	3·03	0·85	3·6	0·000
G46	302	3·93	1·10	3·6	0·001
G50	356	3·40	1·24	2·7	0·004
G51	512	3·23	0·79	4·1	0·005

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

Table 3. Specific mechanical energy and molecular weight results for low protein (11·4%) wheat flour before and after extrusion[a]

Sample	SME (kJ/kg)	Weight avg. mol. wt (MWw) $\times 10^6$	Number avg. mol. wt (MWn) $\times 10^6$	Poly-dispersity ratio (MWw/MWn)	Average lambda
Control	—	5·31	2·35	2·3	0·021
G37	554	2·25	0·74	3·0	0·000
G38	1016	2·20	0·79	2·8	0·000
G41	356	3·34	0·95	3·5	0·001
G42	491	3·32	1·09	3·0	0·000
G47	410	3·62	1·12	3·2	0·001
G48	416	3·41	1·10	3·1	0·001
G49	236	4·34	1·44	3·0	0·002
G52	331	3·79	0·93	4·1	0·009

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

(weight average molecular weight) and MWn (number average molecular weight) values listed in Tables 2 and 3 was conducted. Significant decreases in MWw (for example, from $6{\cdot}53 \times 10^6$ to $2{\cdot}47$–$4{\cdot}10 \times 10^6$ in high protein flour) were observed in all extrudates. Similarly, marked declines of MWn also occurred (Tables 2 and 3). With the exception of one high protein sample (G50), polydispersity ratios (MWw/MWn) all increased as the result of extrusion.

ANOVA showed that die temperature and moisture content were the factors most significantly affecting fragmentation. Consistent with previous studies, fragmentation was promoted at low temperatures (compare MWw at 160°C versus 185°C) and moisture levels (compare 16% versus 20%) (Tables 2 and 3). As observed earlier with wheat using qualitative indices of starch molecular size (Colonna *et al.*, 1984; Davidson *et al.*, 1984), starch fragmentation was more pronounced with decreasing temperature. This was true for both flours, therefore distribution patterns were not affected by differences in protein levels between the two samples. Regression analysis showed that there was a statistically significant inverse relationship between SME and the molecular weight data.

Degree of branching calculations

The parameter λ serves as a qualitative index of average polymer branching frequency and represents the ratio of intrinsic viscosity of branched to linear polymer (Styring *et al.*, 1987; Vilenchik & Ayotte, 1992). Lambda values

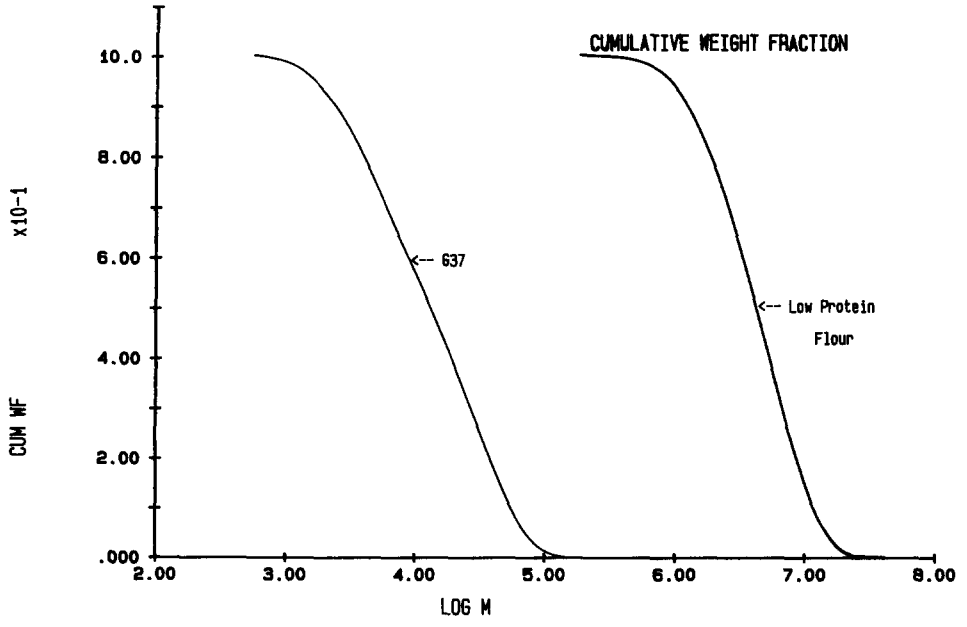


Fig. 3. Plot of cumulative MW fraction (CUM WF) for native low protein wheat flour and extrudate G37.

Table 4. Cumulative molecular weight distributions of high protein (14%) wheat flour before and after extrusion[a]

Sample	MW $\geq 10^7$ (%)	MW 10^7–10^6 (%)	MW 10^6–10^5 (%)	MW 10^5–10^4 (%)
Control	11	73	16	0
G39	0	69	29	2
G40	0	74	24	2
G43	4	71	24	1
G44	3	76	21	0
G45	1	70	27	2
G46	6	72	21	0
G50	3	73	24	0
G51	0	79	15	0

[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. Samples were dissolved in DMAC/LiCl and chromatographed as described in Materials and Methods.

Table 5. Cumulative molecular weight distributions of low protein (11·4%) wheat flour before and after extrusion[a]

Sample	MW $\geq 10^7$ (%)	MW 10^7–10^6 (%)	MW 10^6–10^5 (%)	MW 10^5–10^4 (%)
Control	15	80	5	0
G37	0	66	33	1
G38	0	68	32	0
G41	0	73	27	0
G42	0	74	26	0
G47	4	83	13	0
G48	4	72	24	0
G49	8	73	24	0
G52	0	94	6	0

[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. Samples were dissolved in DMAC/LiCl and chromatographed as described in Materials and Methods.

have been shown to correlate with branching frequencies for various polymers by ^{13}C NMR (Pang & Rudin, 1993). Lambda values (Tables 2 and 3) were dramatically decreased following extrusion, with average λ values declining from 0·021 to less than 0·009, with 15 of the 16 samples below 0·005. Consistent with previous findings for corn (Chinnaswamy *et al.*, 1989; Chinnaswamy & Hanna, 1990) and wheat (Colonna *et al.*, 1984), these values reflect extensive fragmentation of the amylopectin component of the wheat flour.

Methylation analysis

Methylation analysis was previously used to probe extrusion-induced fragmentation of corn starch, and little change in linkage distribution was observed (Rodis *et al.*, 1993). The lack of an increase in t-Glc led to the conclusion that only a small percentage of glycosidic linkages relative to the total number present were affected. Since GPC profiles indicate a more dramatic downshift of molecular weight in wheat, selected samples were subjected to methylation analysis. Methylation analysis was conducted on the two extrudates exhibiting the greatest downshift (G37-11·4% protein and G39-14% protein), and corresponding unprocessed controls. The starch was purified as previously described (Rodis *et al.*, 1993) with recoveries ranging from 53% to 69%.

Table 6. Glycosidic linkage profiles of unprocessed and extruded wheat starch as assessed by methylation analysis[a]

Sample	Glycosidic linkage		Mol. % ± S D 4,6-Glc
	t-Glc	4-Glc	
Low protein control	5·8 ± 0·1	87·1 ± 0·8	4·1 ± 0·3
Low protein extrudate	6·5 ± 0·8	86·9 ± 1·9	4·3 ± 0·6
High protein control	5·9 ± 0·8	87·4 ± 1·8	4·4 ± 0·3
High protein extrudate	6·6 ± 1·5	86·8 ± 0·6	4·3 ± 1·2

[a]Each sample was analyzed three times.

Linkage distributions (Table 6) are in accordance with known structural features of amylopectin (Manners, 1989). In both extrudates, small increases in t-Glc were observed following extrusion. Methylation analysis showed no change in 2,3-Glc and therefore no evidence for the formation of anhydro-linkages (Theander & Westerlund, 1988) was observed (data not reported). Therefore, although GPC clearly documents extensive extrusion-induced fragmentation, the relatively small amount of t-Glc formed indicates cleavage of only a small percentage of linkages. In addition, the absence of glucose or maltodextrin indicates that fragmentation sites are largely internal, fragmenting at the B chains on the amylopectin molecule (Manners, 1989) rather than adjacent to the reducing or non-reducing ends. These results are in agreement with those obtained with corn (Rodis *et al.*, 1993).

Gelatinization characteristics

Samples were reconstituted in 70% water and subjected to differential scanning calorimetry (DSC). In unprocessed samples, one peak (62·7°C and 63·3°C for the low and high protein flours, respectively) was observed which agrees with established gelatinization temperatures for wheat starch (Whistler & Daniel, 1985). Following extrusion, these peaks disappeared, which clearly indicates total loss of ungelatinized starch. These results are consistent with Chinnaswamy *et al.* (1989) and Gomez and Aguilera (1984). Each of these 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 profiles

TPA parameters for high and low protein flours and the extrudates were determined (Table 7). ANOVA of the extrusion conditions with the TPA parameters showed that the interaction of die temperature and moisture content significantly affected all of the TPA parameters (hardness, cohesiveness, springiness, gumminess and chewiness) studied, however this held true only at 20% moisture. For these samples, as temperature was increased, the TPA values decreased. Reasons for the lack of significance at 16% moisture are unclear. In contrast, Halek *et al.* (1989) found that TPA parameters decreased with moisture content using corn meal.

Correlation analysis was used to relate molecular size profiles with TPA values. Changes in MWw (Tables 2 and 3) correlated with springiness, but not with the other TPA parameters evaluated. Whereas die temperature and moisture content significantly affect starch size, strict correlations between the degree of starch fragmentation and textural properties were not observed. Therefore, no simple relationship could be established to link extruder operating conditions or molecular size data with the TPA values of extruded wheat flour.

CONCLUSIONS

Automated GPC with DMAC and LiCl, with application of the universal calibration concept, is a rapid and efficient technique for quantification of starch molecular weight profiles. This procedure readily detects processing-induced modifications of starch size. In contrast to gravity flow GPC with DMSO as the mobile phase, automated GPC provides weight average and number average molecular weight values. In addition, λ values which are indicative of the degree of branching can be obtained. With respect to twin-screw extrusion, fragmentation was promoted at low die temperatures and moisture levels. Methylation analysis, however, did not show markedly increased levels of t-Glc. Although the magnitude of fragmentation in wheat is greater than in corn flours, methylation analysis shows that the number of fragmentation points still represents a very small fraction of glycosidic linkages. Automated GPC in DMAC and LiCl represents a rapid and effective means for monitoring starch molecular size distribution and should gain widespread applicability for structural characterizations of starch.

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The names of companies or commercial products are

Table 7. Texture profile analysis (TPA) parameters of high protein and low protein wheat flour extrudates[a]

Extrudate	Hardness (MPa)	Cohesiveness	Springiness (mn)	Gumminess (MPa)	Chewiness (MPa m)
High protein					
G39	0·18	10·9	6·1	1·9	11·7
G40	0·25	12·2	6·1	3·1	18·7
G43	0·21	9·0	5·2	1·9	9·9
G44	0·20	12·6	6·3	2·5	15·7
G45	0·55	6·9	5·4	3·8	20·5
G46	0·43	9·3	6·9	4·0	27·5
G50	1·11	11·9	6·2	13·2	82·2
G51	4·86	17·8	7·3	86·7	628·0
Low protein					
G37	0·21	7·4	3·9	1·6	6·2
G38	0·15	7·5	4·8	1·1	5·4
G41	0·14	11·9	5·8	1·7	9·7
G42	0·14	6·4	4·0	0·9	3·7
G47	2·24	11·0	7·2	24·8	178·6
G48	0·69	7·1	6·0	4·9	29·5
G49	1·23	8·7	6·5	10·7	70·2
G52	3·24	12·1	7·4	39·3	290·7

[a]TPA parameters were obtained according to the method of Halek *et al.* (1989) and Bourne and Comstock (1981).

given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the United States Department of Agriculture over others not mentioned.

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