Starch Fragmentation and Protein Insolubilization During Twin-Screw Extrusion of Corn Meal

LU-FANG WEN,[1] PANAYOTIS RODIS,[2] and BRUCE P. WASSERMAN[1,3]

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ABSTRACT

Degermed corn meal was extruded under 15 different extrusion conditions in a twin-screw extruder. Variables included moisture content (20, 25, and 30%), screw speed (100, 200, and 300 rpm), and temperature (100, 150, and 200°C). Extrudates were dissolved in dimethly sulfoxide (DMSO). Carbohydrate and protein were analyzed for solubility and molecular size changes. Carbohydrate solubility was not significantly affected by extrusion; however, protein solubility significantly decreased. Fragmentation patterns of the extruded starches were related to variables by response surface analysis. High molecular weight polysaccharide decreased as moisture content and temperature were decreased and as screw speed was raised. Protein became more resistant to solubilization in DMSO after extrusion. Sodim dodecyl sulfate-polyacrylamide gel electrophoresis indicated DMSO-soluble proteins did not fragment in an analogous manner as carbohydrate and also were not modified to any significant extent.

Extrusion technology has been extensively applied to produce many novel starch- or protein-based foods. During twin-screw extrusion, the formulation is introduced into the heated screw barrel of the extruder, heated, transported, compressed by the rotating screws, and pumped through the die at high temperature and pressure. The combination of shearing, temperature, and pressure creates many opportunities for molecular changes in carbohydrates, proteins, and lipids.

With respect to carbohydrate, it is well established that the starch fraction of wheat (Davidson et al 1984) and corn (Chinnaswamy and Hanna 1990) undergoes a certain degree of fragmentation during extrusion cooking. The effects of extrusion on starch structure have been studied by a number of investigators using both direct and indirect methods. Indirect measurements consistent with starch fragmentation include decreased viscosity (Owusu-Ansah et al 1983, Diosady et al 1985), decreased water adsorption index (Mercier and Feillet 1975, Williams et al 1977, Owusu-Ansah et al 1983), increased amylase susceptibility (Gomez and Aguilera 1984), and increased degree of gelatinization (Gomez and Aguilera 1984). Direct measurements documenting starch fragmentation in wheat and corn starch by gel filtration chromatography have been reported by a number of investigators (Colonna et al 1984, Davidson et al 1984, Chinnaswamy and Hanna 1990). The characteristic pattern observed by each of these groups was a decrease in high molecular weight material and a corresponding increase in lower molecular weight polysaccharide. Scanning electron microscopy was used to characterize surface changes (Gomez and Aguilera 1984).

The effects of extrusion on protein structure are less well understood (Stanley 1989). Racicot et al (1981) showed that the solubility of corn meal protein in ethanol and alkali is significantly decreased after extrusion. Cumming et al (1973) suggested that exposure to heat and pressure affects free sulfhydryl groups and disulfide bonds in soy protein, which results in insolubilization. However, Burgess and Stanley (1976) suggested that disulfide bonds play only a minor role in extrusion texturization and proposed that intermolecular amide bonds are responsible for texture formation. Later, Hager (1984) and Neumann et al (1984) showed that the intermolecular disulfide bonding in soy is important and found no evidence for appreciable intermolecular peptide bond formation. Microstructural changes that occur during extrusion of soy, such as plasticization and fiber formation, have been documented (Gwiazda et al 1987). In an unprocessed corn zein fraction, Abe et al (1985) demonstrated the existence of intermolecular disulfide bonds.

The major objective of this study was to examine the effects of extrusion conditions on the molecular size of starch and protein in a corn meal system. Conditions studied were moisture content, temperature, and screw speed. To accomplish this objective, corn meal extrudates were solubilized in dimethylsulfoxide (DMSO), and analyzed by gel filtration chromatography and sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Results were interpreted by response surface analysis. The effects of extrusion on lipids in corn meal were recently described by Izzo and Ho (1989).

MATERIALS AND METHODS

Materials

Corn meal was obtained from Lauhoff Grain Co., Danville IL. Analysis data provided by the company indicated the following proximate composition: 12% moisture, 7% protein, 0.7% oil, 0.5% fiber, 0.4% ash, and 79.4% nitrogen-free extract. DMSO (99.9%) was purchased from Aldrich Chemical Co. Milwaukee, WI. Sephacryl S-1000 was from Pharmacia, Piscataway, NJ.

Extrusion Conditions

Extrusion of corn meal was performed in a Werner & Pfleiderer corotated twin-screw extruder (model ZSK-30) under 15 sets of selected operating conditions: barrel temperature, 100, 150, and 200°C; screw speed, 100, 200, and 300 rpm; and feed moisture, 20, 25, and 30%. Table I summarizes conditions for each test run.

TABLE I
Extrusion Conditions for Response Surface Analysis

Sample No.	Moisture Content (%)	Barrel Temperature (°C)	Screw Speed (rpm)
1	30	200	200
2	25	200	100
3	25	200	300
4	20	200	200
5	25	150	200
6	30	100	200
7	25	100	300
8	25	100	100
9	20	100	200
10	25	150	200
11	30	150	100
12	30	150	300
13	25	150	200
14	20	150	100
15	20	150	300

[1]Department of Food Science, New Jersey Agricultural Experiment Station, Cook College, Rutgers University, New Brunswick 08903.
[2]Present address: Agricultural College of Athens, Dept. of Food Science and Technology, Votanikos, Athens, 11855, Greece.
[3]To whom correspondence should be addressed.

TABLE II
Relative Recovery of Carbohydrate and Protein in DMSO and I_2-Binding Capacity[a]

Sample	Carbohydrate Recovered (%)[b]		Protein Recovered (%)[c]			I_2-Binding Capacity
	Total (% A_{490})	Peak I (% A_{490})	Total (% A_{595})	Peak I (% A_{280})	Peak II (% A_{280})	(% A_{620})
Corn meal	100.0	100.0	100.0	100.0	100.0	100
1	99.0	86.0	88.2	49.6	6.6	118
2	94.1	80.9	79.4	26.2	5.2	120
3	93.3	75.7	86.2	22.6	3.4	131
4	107.7	47.4	73.3	8.2	13.7	100
5	100.5	65.0	76.1	31.5	6.3	108
6	101.7	78.1	97.5	29.7	5.4	114
7	93.9	40.3	102.9	33.0	27.2	112
8	98.1	56.9	77.1	47.9	55.9	112
9	114.9	45.1	90.7	43.3	49.6	131
10	112.3	63.4	69.4	9.2	6.6	112
11	106.3	77.2	84.6	22.7	12.7	118
12	114.2	65.2	71.9	41.1	17.5	124
13	110.1	56.1	76.6	29.5	14.0	131
14	104.0	65.2	77.0	30.5	48.5	120
15	116.7	36.6	73.5	24.2	14.7	126
Mean (1–15)	104.5	62.6	81.6	32.7	20.5	118

[a]Samples were dissolved into dimethylsulfoxide (DMSO) as described in Materials and Methods. "Total" refers to total amount of carbohydrate or protein solubilized in DMSO relative to unextruded corn meal as assayed by the phenol-sulfuric acid assay or Bradford assay, respectively. Peak I refers to the relative recovery of the high molecular weight (void volume) fraction after gel filtration chromatography (Fig. 2). Peak II, the low molecular weight fraction, is also defined in Fig. 2. Carbohydrate in peak I was assayed by the phenol-sulfuric acid assay and protein in peaks I and II by absorbance at 280 nm.
[b]Average of four analyses.
[c]Average of at least five analyses.

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.

Solubilization

Samples (10 mg) were suspended in 3 ml of 100% DMSO and stirred at room temperature for 72 hr. Samples were centrifuged at 10,000 × *g* for 30 min to recover DMSO-soluble starch and protein. Supernatants were assayed for soluble carbohydrate by the phenol-sulfuric acid assay (Dubois et al 1956). Protein was assayed by measuring absorbance at 280 nm or by the Coomassie blue dye-binding method (Bradford 1976).

Gel Filtration Chromatography

Supernatants were diluted to 50% DMSO and centrifuged at 10,000 × *g* for 30 min. Aliquots of 1.5 ml were chromatographed on a 0.9 × 55 cm column packed with Sephacryl S-1000. The column was eluted with 50% DMSO at a flow rate of 5 ml/hr. Fractions of 1.4 ml were collected and assayed for carbohydrate by the phenol sulfuric acid assay (Dubois et al 1956) and for protein by measuring absorbance at 280 nm with an on-line ultraviolet (UV) monitor. Amylose was assayed colorimetrically (Knutson 1986).

I_2-Binding Capacity

Samples (10 mg) were suspended in 3 ml of 100% DMSO, stirred at room temperature for 72 hr, and centrifuged at 10,000 × *g* for 30 min. Supernatants were assayed for I_2-binding capacity colorimetrically (Knutson 1986).

SDS Polyacrylamide Gel Electrophoresis

Proteins in corn meal and column fractions were electrophoresed in 9–18% polyacrylamide gradient gels using the procedure of Laemmli (1970) as modified by Porzio and Pearson (1976). Protein bands were visualized by silver staining (Merril et al 1984).

Response Surface Methodology

The percentage of carbohydrate recovered in the the large

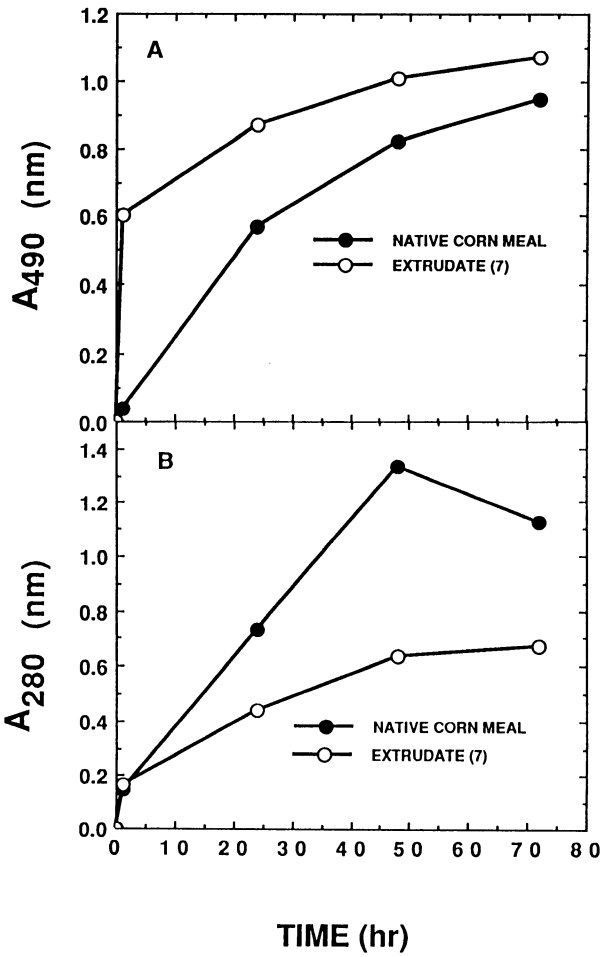

Fig. 1. Time course of carbohydrate and protein solubilization. **A,** Carbohydrate solubility. **B,** Protein solubility. Native corn meal (-●-) and extrudate 7 (-○-) were solubilized at room temperature in 100% dimethylsulfoxide and sampled at various times as described in Materials and Methods.

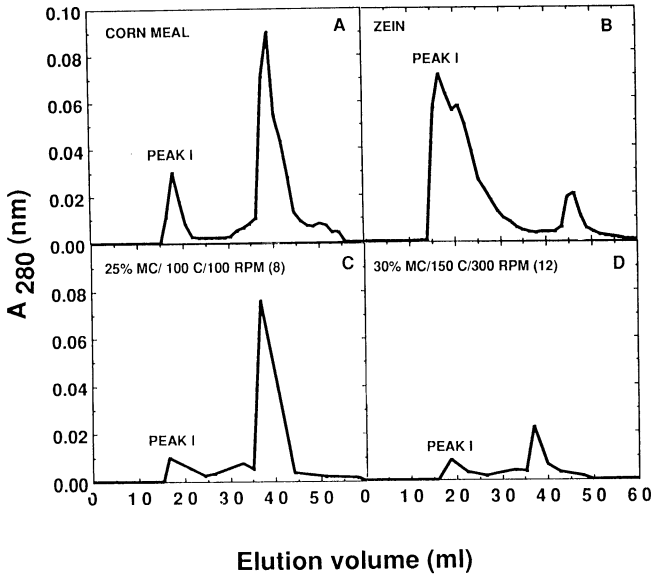

Fig. 2. Molecular size profiles of dimethylsulfoxide-soluble protein from **A**, corn meal. **B**, zein. **C**, Extrudate 8. **D**, Extrudate 12.

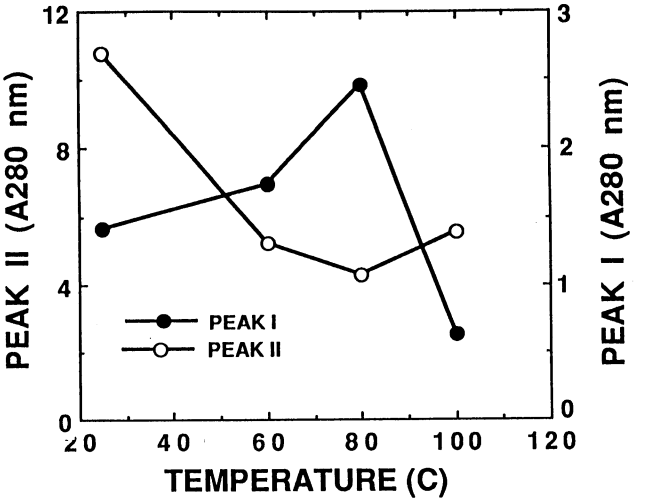

Fig. 3. Effect of temperature on the amount of A_{280}-absorbing material in peaks I and II. Corn meal was solubilized for 2 hr in dimethylsulfoxide as described in Materials and Methods. Solubilized material was then fractionated by gel filtration chromatography. Peaks I (–●–) and II (–○–) were then quantitated by calculation of the amount of A_{280}-absorbing material under each peak.

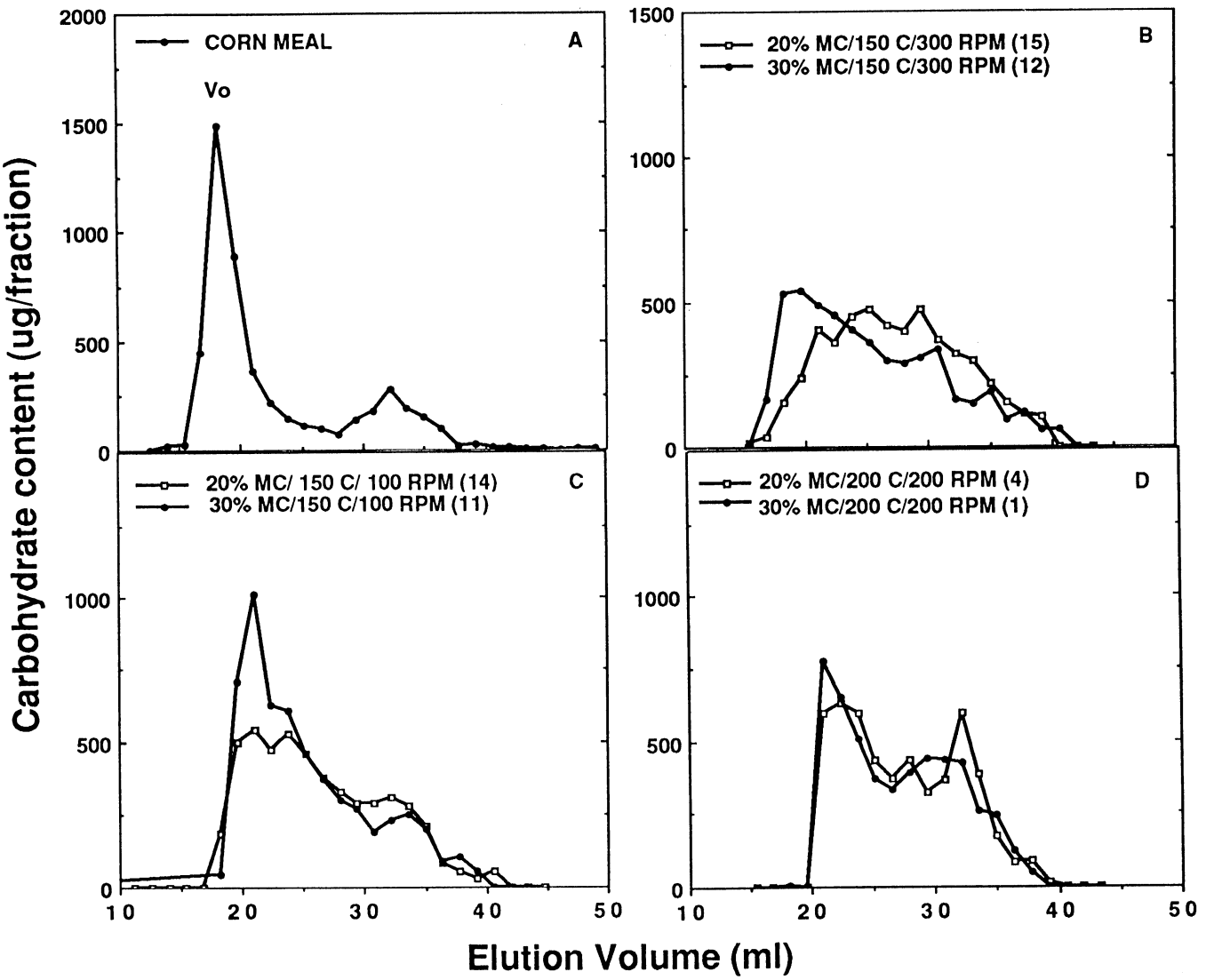

Fig. 4. Effect of feed moisture content on polysaccharide molecular size distribution. **A**, Corn meal. **B**, Extrudates 15 and 12. **C**, Extrudates 14 and 11. **D**, Extrudates 4 and 1.

molecular size fraction (peak I) of chromatograms was related to extrusion variables by a response surface method using the SAS program for a three-component system (SAS 1985). Response surface curves were plotted on a Hewlett-Packard plotter using Display Integrated Software System and Plotting Language (DISSPLA).

RESULTS

Degermed corn meal was extruded under 15 different extrusion conditions (Table I). Variables included moisture content (20, 25, and 30%), screw speed (100, 200, and 300 rpm) and temperature (100, 150, and 200°C). The effects on the solubility and molecular size profiles of polysaccharide and protein were ascertained.

Solubilization of Starch and Protein

DMSO is an effective solubilizer of starch (Killion and Foster 1960, Leach and Schoch 1962, Carpita and Kanabus 1987). A time course of carbohydrate solubilization from corn meal and extrudate 7 is shown in Figure 1A. Carbohydrate from the extruded sample dissolved at a significantly faster rate than from corn meal, with a dramatic difference in the amount of material solubilized during the first 2 hr. This effect was consistently observed in all extrudates and is suggestive of the occurrence of fragmentation. As equilibrium was approached, the amount of carbohydrate solubilized did not differ significantly between corn meal and extrudates (Fig. 1A). Faster rates of solubilization were also consistently observed with extruded amylopectins and amyloses (P. Rodis, *unpublished results*).

The I_2-binding capacity of the DMSO extract was measured (Table II). Increased I_2-binding capacity was observed in all extrudates except sample 4. Since I_2-binding should correlate with amylose content, these data suggest that the amount of linear polysaccharide increased after extrusion. This is indicative of fragmentation.

The behavior of protein in DMSO was very different from carbohydrate. One apparent difference was that extrusion decreased the rate of solubilization of A_{280}-absorbing material after the first 2 hr of solubilization (Fig. 1B).

The exact decrease in total protein recovery was difficult to quantitate in DMSO extracts by direct assay of A_{280}-absorbing material. For example, Figure 1B shows a 50% decrease in A_{280}-absorbing material. However, gel filtration chromatography of corn meal (Fig. 2A) shows that a low-molecular weight peak of A_{280}-absorbing material (peak II) was removed during zein purification (Fig. 2B). Presuming that most of the protein in corn meal is zein, this indicated that corn meal may contain nonprotein-aqueous UV-absorbing material that could interfere with protein measurement.

The effect of solubilization temperature on the solubilization rate of each peak was investigated (Fig. 3). Whereas the size of peak I increased as solubilization temperature was raised to 80°C, the size of peak II declined dramatically. Between 80 and 100°C the size of peak I decreased, whereas the size of peak II showed a slight increase. Thus, peaks I and II respond in opposite ways to solubilization temperature. This could indicate the occurrence of cross-linking as solubilization temperatures are increased from 20 to 80°C.

The best index of protein recovery was quantitation of high molecular size A_{280}-absorbing material (peak I). Figures 2C and D, which depict typical elution profiles of DMSO-solubilized extrudates, show greatly decreased recovery of peak I protein, relative to corn meal. Mean recovery was 33%. The decline in peak I protein did not correlate with an increase in peak II (Fig. 2C and 2D, Table II), which would have been expected if the polypeptides comprising peak I were being fragmented. Therefore, this result suggests the occurrence of insolubilization rather than fragmentation. The mean recovery of peak II was only 21% (Table II).

Measurement of total DMSO-soluble protein by a second method, Coomassie blue dye-binding, confirmed the decrease in protein recovery (Table II). In 12 of the 15 samples, protein recovery declined by more than 10%. Mean protein recovery was 82%. The possible contribution of impurities to absorbance readings is unknown.

Effect of Extrusion Parameters on Apparent Molecular Size

Carbohydrate profiles. Native corn meal and the 15 extruded samples were analyzed for polysaccharide size distribution by gel filtration chromatography. The carbohydrate profile of native corn meal gave two peaks (Figs. 4A, 5A, and 6A). The bulk of material at the volume (peak I) coeluted with pure amylopectin. Based on this observation and previous work (Biliaderis et al

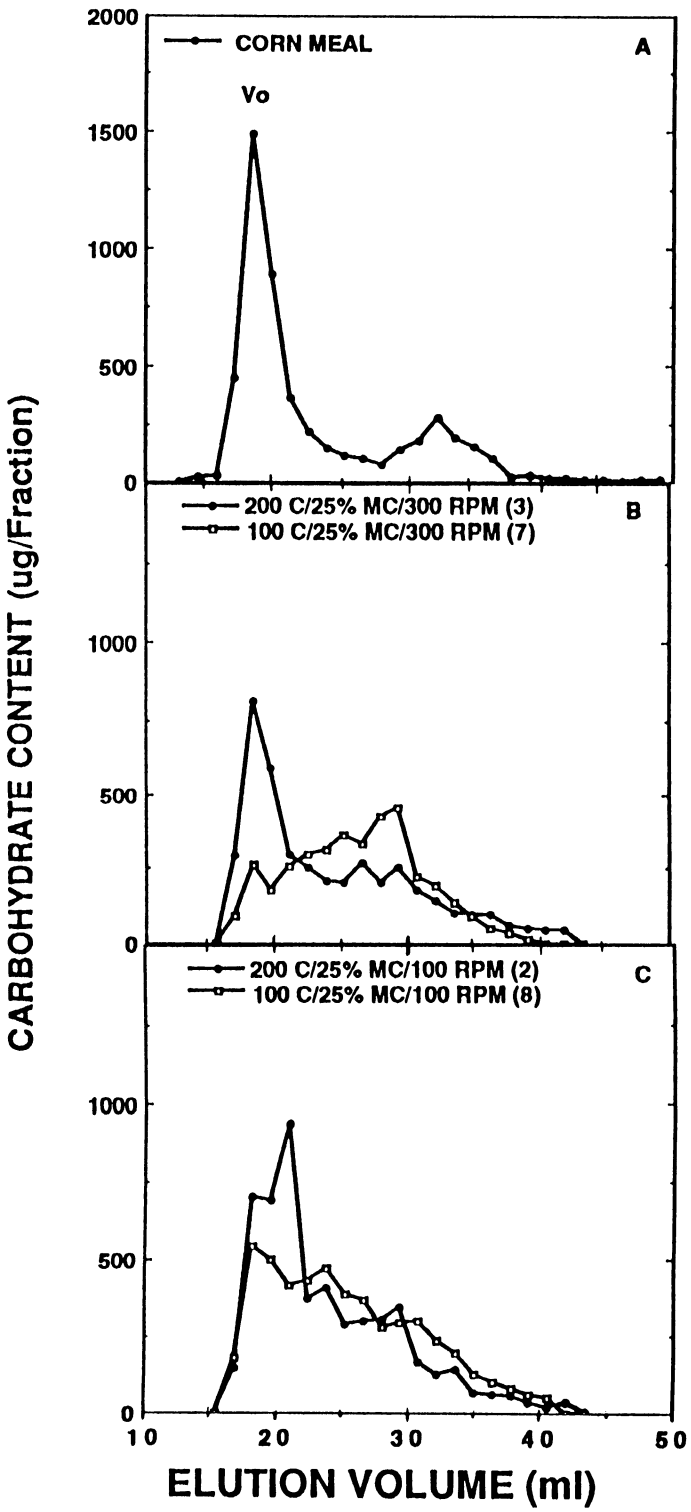

Fig. 5. Effect of temperature on polysaccharide molecular size distribution. **A**, Corn meal. **B**, Extrudates 3 and 7. **C**, Extrudates 2 and 8.

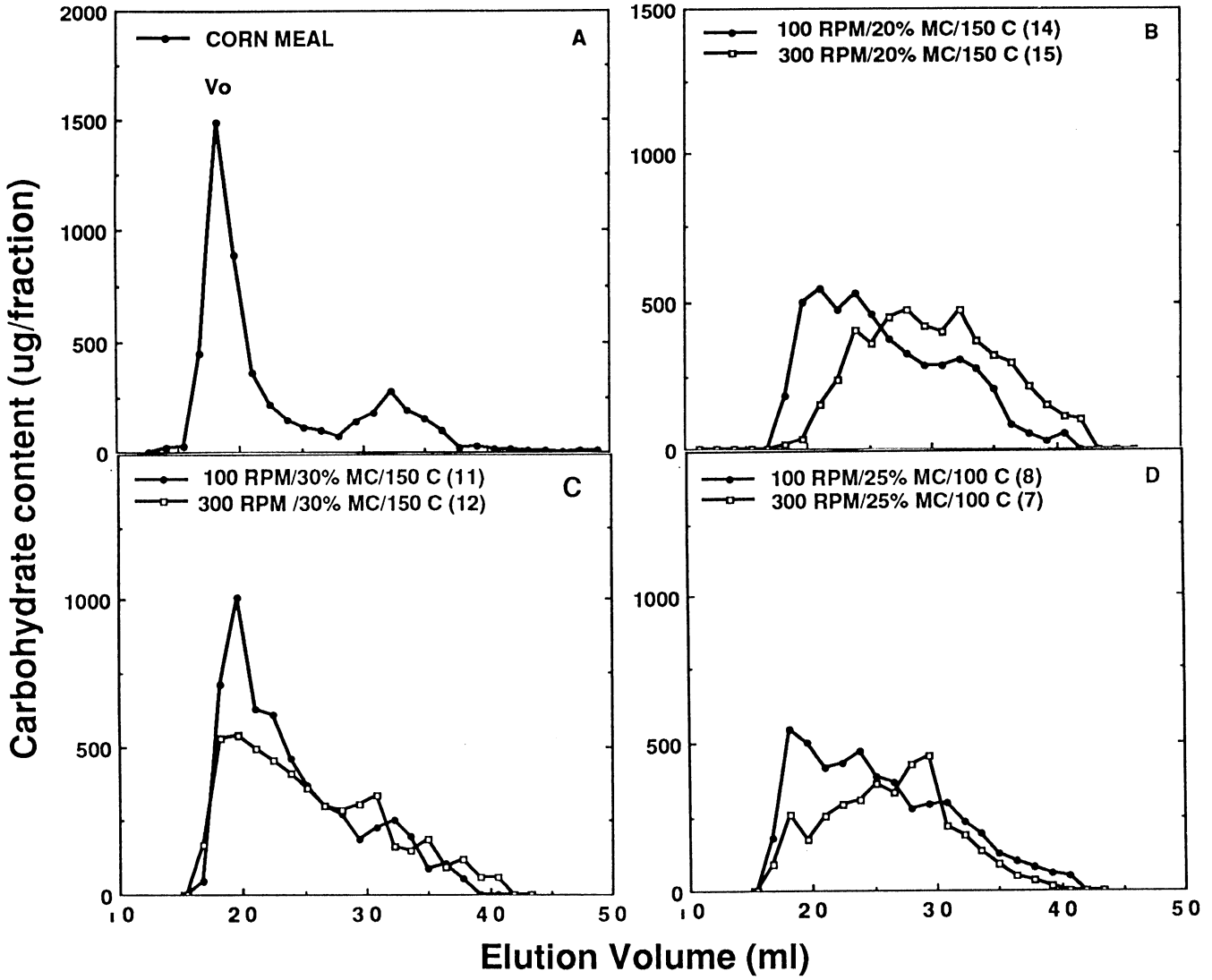

Fig. 6. Effect of screw speed on polysaccharide molecular size distribution. **A,** Corn meal. **B,** Extrudates 14 and 15. **C,** Extrudates 11 and 12. **D,** Extrudates 8 and 7.

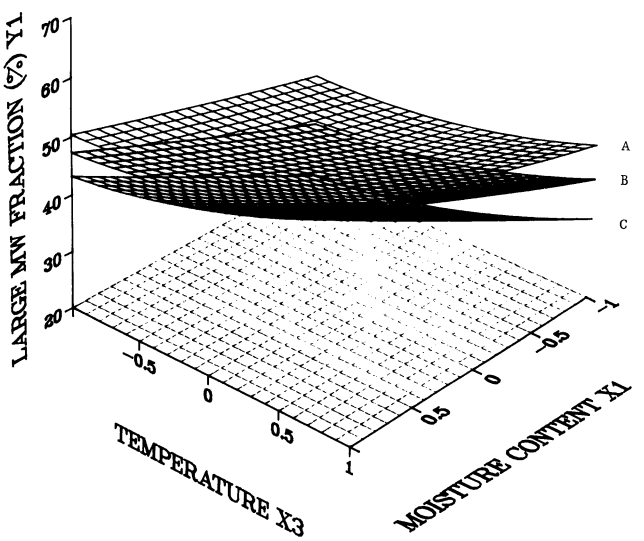

Fig. 7. Response surface analysis of peak I carbohydrate. Large molecular weight fraction (% *Y*1) vs. moisture content (*X*1) and temperature (*X*3) at screw speed *X*2: **A** = 100 rpm, **B** = 200 rpm, and **C** = 300 rpm.

1979, Biliaderis et al 1981, Chinnaswamy and Hanna 1990), it was concluded that peak I is amylopectin. The smaller peak that is retained by the column (peak II) formed a blue complex with I_2 and is therefore amylose. Peak I represented 68% of the material eluting from the column. Under all extrusion conditions tested, a reduction of peak I was observed. Reductions in the size of peak I were accompanied by peak broadening and extensive tailing. The relative amount of large molecular weight material decreased from 68% to a range of 24–58% after extrusion. Some paired comparisons showing the effects of varying selected extrusion conditions on size distribution are shown in Figures 4–6.

Effects of feed moisture content are shown in Figure 4. At each screw speed and temperature tested, a decrease in moisture content from 30 to 20% resulted in increased fragmentation. At medium temperature (150°C) and high screw speed (300 rpm), peak I decreased from 44 to 25% as moisture decreased from 30 to 20% (Fig. 4B). At 150°C and low screw speed (100 rpm), peak I decreased from 52 to 40% as moisture decreased from 30 to 20% (Fig. 4C). At high temperature (200°C) and medium screw speed (200 rpm), peak I decreased from 58 to 32% as moisture decreased from 30 to 20% (Fig. 4D). These data also indicated that starch fragmentation is not only influenced by moisture content.

Representative chromatograms showing the effect of temperature on polysaccharide size distribution are shown in Figure

5. In general, fragmentation tended to increase as temperature was lowered.

Screw speed also had a direct effect on polysaccharide size distribution. For example, screw speeds of 300 rpm resulted in more fragmentation than at 100 rpm (Fig. 6A–D).

The response surface analysis of Figure 7 integrates the effects of temperature, screw speed, and moisture content. $Y1$ is the percentage of polysaccharide recovered in the high molecular size (peak I) fraction relative to the total amount of material recovered in each chromatogram. In other words, as fragmentation occurs, $Y1$ decreases. It shows that fragmentation increases as moisture content and temperature are decreased and as screw speed is raised.

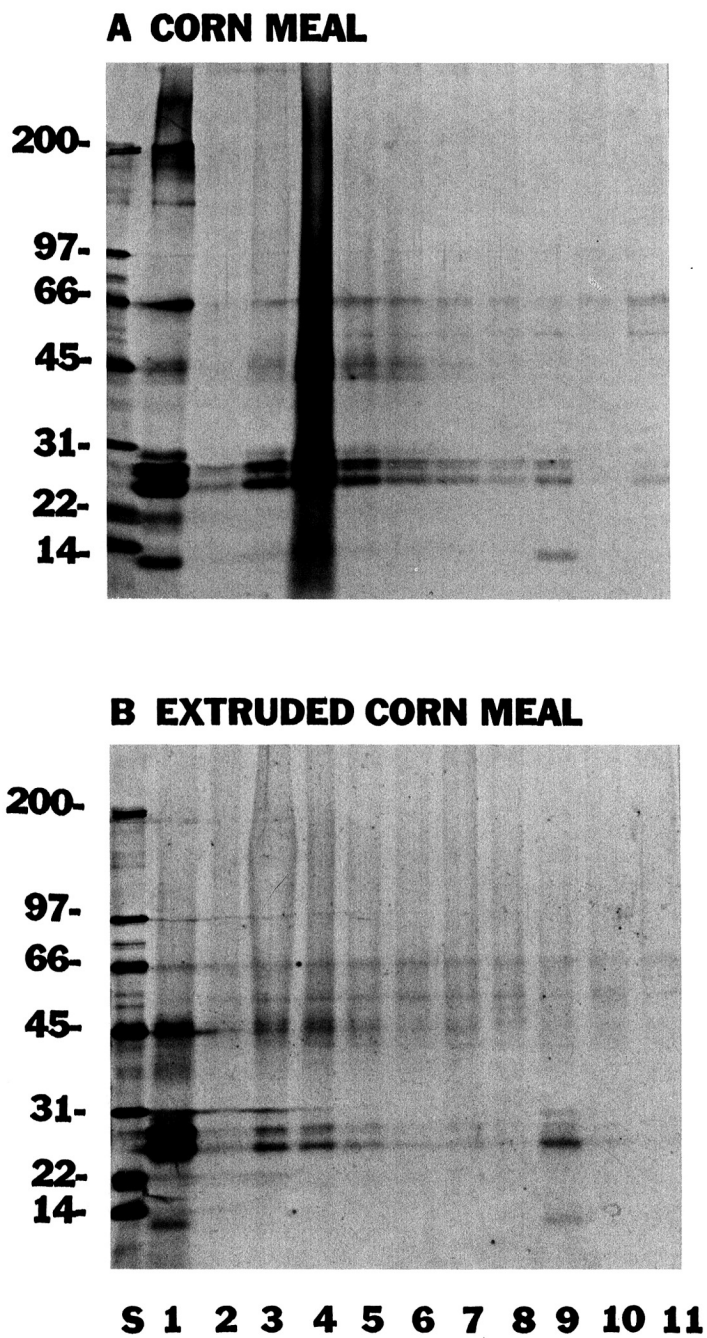

Fig. 8. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of column fractions of native and extruded corn meal. **A,** Corn meal. **B,** Extrudate 4. One milliliter of corn meal and extrudate 4 (3.3 mg/ml in dimethylsulfoxide) was lyophilized, and the residue was redissolved into 90 μl of SDS sample buffer containing 3% SDS, 10% glycerol, 62.5 m*M* Tris and 5% 2-mercaptoethanol (lane 1). Column fractions (2.8 ml) were lyophilized and treated the same as corn meal (peak I, lanes 2–7; peak II, lanes 8–11).

These results indicate that both mechanical forces and thermal effects play an important role in starch fragmentation. Thus, the relationships observed in a corn meal system are consistent with results recently obtained with amylose-amylopectin systems in a single-screw extruder (Chinnaswamy and Hanna 1990).

The equation describing this response surface model is:

$$Y1 = 41.74 + 9.14\,X1 - 5.05\,X2 + 6.03\,X3 + 0.21\,X1^2 + 2.82\,X1X2 - 0.51\,X2^2 + 0.18\,X1X3 + 1.41\,X2X3 + 2.34\,X3^2$$

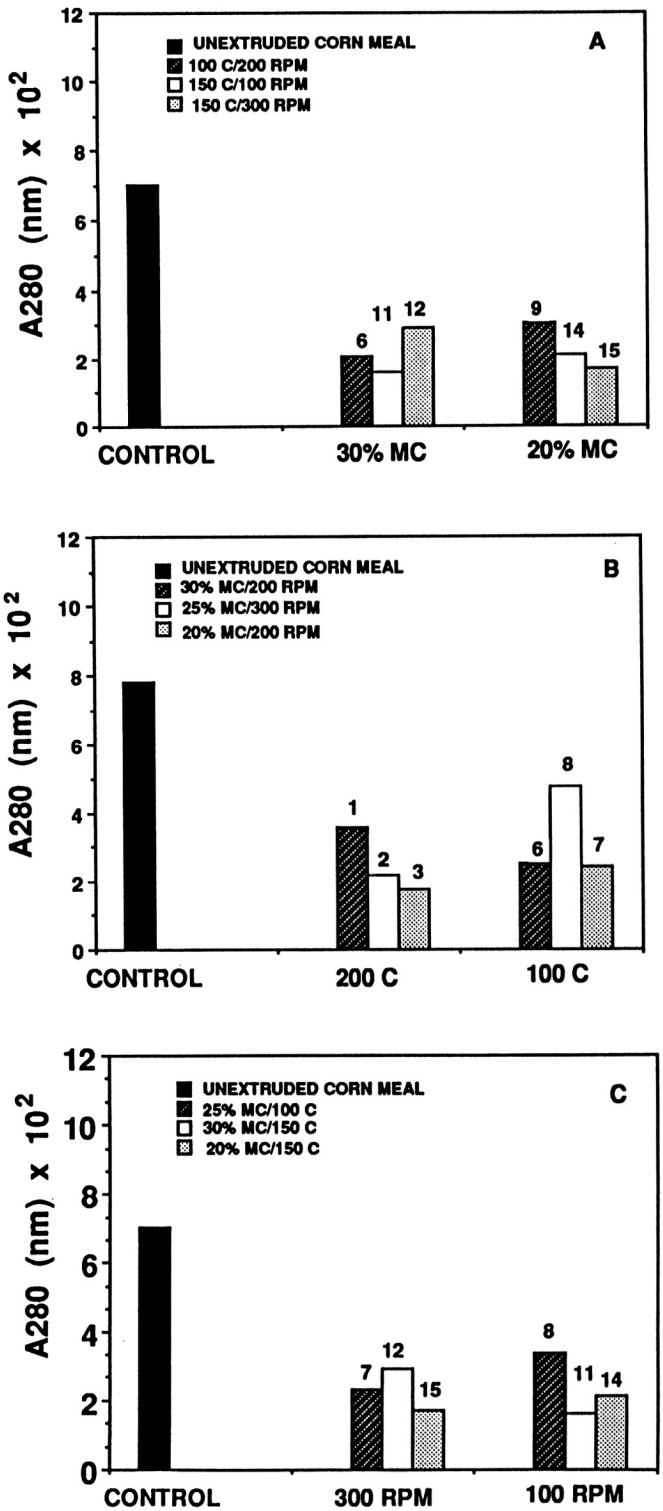

Fig. 9. Effect of moisture content, temperature, and screw speed on recovery of peak I protein. Samples were dissolved and chromatographed as described in Materials and Methods.

It gave a correlation coefficient (r^2) of 0.87 at a probability level of 0.1. The three linear terms (moisture content ($X1$), screw speed ($X2$), and temperature ($X3$) gave maximal contributions to Y1. Of the three linear terms, moisture content had the greatest effect on the decrease of peak I. The greatest interaction was between moisture content and screw speed. Moreover, interactions between temperature and screw speed, and moisture and temperature also affected peak I. This surface predicts maximal fragmentation at low temperature and moisture and at high screw speed.

Protein profiles. A_{280} tracings did not show any evidence of degradation. Unlike carbohydrate, the loss of peak I was not accompanied by tailing nor by an increase in material of smaller molecular size. Figure 2C and D are two representative chromatograms.

Additional proof that protein did not undergo fragmentation of a covalent nature was obtained by SDS polyacrylamide gel electrophoresis (Fig. 8). Gel filtration fractions from native corn meal (Fig. 8A) and sample 4 (Fig. 8B) were dried, suspended in SDS sample buffer containing 2-mercaptoethanol, and analyzed. Polypeptides of 200, 150, 66, 44, 28, 26, 24, 22, and 9 kDa were observed, in agreement with Paulis and Bietz (1988). They reported that the polypeptides at 66 and 44 kDa were trimeric and dimeric forms of the 22 kDa polypeptide.

No differences in polypeptide banding patterns of corn meal and the extruded sample were observed. Most protein was recovered in peak I. The absence of new polypeptides after extrusion provides evidence that DMSO-soluble protein undergoes negligible, if any, covalent breakages in the polypeptide backbone. The fact that the individual polypeptides did not increase in apparent molecular size suggests that DMSO-soluble protein does not become covalently modified (e.g., glycosylation by low molecular weight amylopectin fragmentation products) to any significant degree.

An attempt was made to correlate recovery of peak I protein with extrusion parameters (Fig. 9). It did not appear that moisture content (Fig. 9A) or temperature (Fig. 9B) had a significant effect on peak I recovery. There may have been a slight trend towards decreased recovery at high screw speeds (Fig. 9C); however, to confirm a distinction, additional replicates with appropriate statistical analysis will be required. The major effect of extrusion on DMSO-soluble protein, therefore, appears to be insolubilization.

DISCUSSION

This study investigated the effects of extrusion conditions on the solubility and molecular size of corn meal polysaccharide and protein. Maximal fragmentation generally occurred at low moisture content (20%), low temperature (100° C), and high screw speed (300 rpm). Under these conditions, mechanical energy is at its peak and starch tends to remain viscous. Ultimately, this leads to extreme pressures in the die and promotion of fragmentation by shearing effects.

Previous papers have referred to the phenomenon of starch cleavage as "degradation." We believe that the term "fragmentation" is a more accurate description, because these data are consistent with breakage into fragments rather than chemical breakdown.

Our results are in accordance with the potato starch extrusion study of Senouci et al (1986). On the other hand, Williams et al (1977) reported maximal water absorption and dextrinization at high temperature and low moisture content in extruded yellow corn grits. The current study predicts enhanced fragmentation at low temperature, low moisture content, and high screw speed.

In contrast to starch, the size distribution of DMSO-soluble protein was unchanged by extrusion. However, significant amounts of protein became insolubilized. This observation is consistent with previous studies demonstrating reductions of protein solubility as a function of temperature in soy (Hager 1984) and corn meal protein (Racicot et al 1981). Thermal denaturation of corn meal protein may lead to oxidation of the sulfur-containing amino acids cysteine and methionine (Evans and Butts 1949). Tyrosine, serine, and threonine are also destroyed under severe conditions (Means and Feeney 1971). These effects were observed with extruded rice protein (Noguchi et al 1982) and ethanol-soluble wheat protein (Theander and Westerlund 1988). It is interesting to note that hexane-extractable lipid also decreased following extrusion (Izzo and Ho 1989).

On the surface it may appear that the extent of starch fragmentation is greater in a corn meal system, which contains lipids and proteins, than with pure amylopectin (Colonna and Mercier 1983, Davidson et al 1984, P. Rodis, *unpublished data*). However, this observation may relate to the fact that single-screw extruders were used. Colonna and Mercier (1983) reported decreased starch fragmentation in the presence of added lipid. The effect of protein and lipid on starch fragmentation in this twin-screw system remains to be elucidated and will be the subject of further study.

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LITERATURE CITED

ABE, M., MIYASAKA, S., and ARAI, S. 1985. Analyses of sulfhydryl groups in zein. Agric. Biol. Chem. 49:1415-1420.

BILIADERIS, C. G., GRANT, D. R., and VOSE, J. R. 1979. Molecular weight distributions of legume starches by gel chromatography. Cereal Chem. 56:475-480.

BILIADERIS, C. G., GRANT, D. R., and VOSE, J. R. 1981. Structural characterizations of legume starches. I. Studies on amylose, amylopectin, and beta-limit dextrins. Cereal Chem. 58:496-502.

BRADFORD, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principles of protein dye-binding. Anal. Biochem. 72:143-147.

BURGESS, L. D., and STANLEY, D. W. 1976. A possible mechanism for thermal texturization of soybean protein. Can. Inst. Food Sci. Technol. J. 9:228-231.

CARPITA, N. C., and KANABUS, J. 1987. Extraction of starch by dimethyl sulfoxide and quantitation by enzymatic assay. Anal. Biochem. 161:132-139.

CHINNASWAMY, R., and HANNA, M. A. 1990. Macromolecular and functional properties of native and extrusion-cooked corn starch. Cereal Chem. In press.

COLONNA, P., and MERCIER, C. 1983. Macromolecular modifications of manioc starch components by extrusion-cooking with and without lipids. Carbohydr. Polym. 3:87-107.

COLONNA, P., DOUBLIER, J. L., MELCION, J. P., DEMONREDON, F., and MERCIER, C. 1984. Extrusion cooking and drum drying of wheat starch. I. Physical and macromolecular modifications. Cereal Chem. 61:538-543.

CUMMING, D. B., STANLEY, D. W., and DEMAN, J. M. 1973. Fate of water soluble soy protein during thermoplastic extrusion. J. Food Sci. 38:320-323.

DAVIDSON, V. J., PATON, D., DIOSADY, L. L., and LAROCQUE, G. 1984. Degradation of wheat starch in a single screw extruder: Characteristics of extruded starch polymers. J. Food Sci. 49:453-458.

DIOSADY, L. L., PATON, D., ROSEN, N., RUBIN, L. J., and ATHANASSOULIAS, C. 1985. Degradation of wheat starch in a single-screw extruder: Mechano-kinetic breakdown of cooked starch. J. Food Sci. 50:1697-1699.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

EVANS, R. J., and BUTTS, H. A. 1949. Inactivation of amino acids by autoclaving. Science 109:569-571.

GOMEZ, M. H., and AGUILERA, J. M. 1984. A physicochemical model for extrusion of corn starch. J. Food Sci. 49:40-43.

GWIAZDA, S., NOCUCHI, A., and SAIO, K. 1987. Microstructural studies of texturized vegetable protein products: Effects of oil addition and transformation of raw materials in various sections of a twin screw extruder. Food Microstruct. 6:57-61.

HAGER, D. F. 1984. Effects of extrusion upon soy concentratrate solubility. J. Agric. Food Chem. 32:293-296.

IZZO, M. T., and HO, C.-T. 1989. Protein-lipid interaction during single-screw extrusion of zein and corn oil. Cereal Chem. 66:47-51.
KILLION, P. J., and FOSTER, J. F. 1960. Isolation of high molecular amylose by dimethylsulfoxide dispersion. J. Polym. Sci. 46:65-73.
KNUTSON, C. A. 1986. A simplified colorimetric procedure for determination of amylose in maize starches. Cereal Chem. 63:89-92.
LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.
LEACH, H. W., and SCHOCH, T. J. 1962. Structure of the starch granule. III. Solubilities of granular starches in dimethyl sulfoxide. Cereal Chem. 39:318-327.
MEANS, G. E., and FEENEY, R. E. 1971. Chemical modifications of proteins. Holden-Day, Inc.: San Francisco, CA.
MERCIER, C., and FEILLET, P. 1975. Modification of carbohydrate components by extrusion-cooking of cereal products. Cereal Chem. 52:283-298.
MERRIL, C. R., GOLDMAN, D., and VAN KEUREN, M. L. 1984. Gel protein stains: Silver stain. Methods Enzymol. 104:441-447.
NEUMANN, P. E., JASBERG, B. K., WALL, J. S., and WALKER, C. E. 1984. Uniquely textured products obtained by co-extrusion of corn gluten meal and soy flour. Cereal Chem. 61:438-445.
NOGUCHI, A., KUGIMIYA, W., HAQUE, Z., and SAIO, K. 1982. Physical and chemical characteristics of extruded rice flour fortified with soybean protein isolate. J. Food Sci. 47:240-245.
OWUSU-ANSAH, J., VAN DE VOORT, F. R., and STANLEY, D. W. 1983. Physicochemical changes in corn starch as a function of extrusion variables. Cereal Chem. 60: 319-324.
PAULIS, J. W., and BIETZ, J. A. 1988. Characterization of zein fractionated by reversed-phase high-performance liquid chromatography. Cereal Chem. 65:215-222.
PORZIO, M. A., and PEARSON, A. M. 1977. Improved resolution of myofibrillar proteins with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Biochim. Biophys. Acta 490:27-34.
RACICOT, W. F., SATTERLEE, L. D., and HANNA, M. A. 1981. Interaction of lactose and sucrose with cornmeal proteins during extrusion. J. Food Sci. 46:1500-1506.
SAS INSTITUTE. 1985. SAS/STAT Guide for Personal Computers, Version 6 Edition. SAS Institute Inc.: Cary, NC. 378 pp.
SENOUCI, A., and SMITH, A. C. 1986. The extrusion cooking of potato starch material. Starch/Staerke 38:78-82.
STANLEY, D. W. 1989. Protein reactions during extrusion processing. Pages 321-341 in: Extrusion Cooking: The Theory and Practice of Food Extrusion. C. Mercier, P. Linko, and J. M. Harper, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.
THEANDER, O., and WESTERLUND, E. 1988. The effects on aqueous ethanol-soluble carbohydrates and protein in heat-processed whole grain wheat and white flour. J. Cereal Sci. 7:145-152.
WILLIAMS, M. A., HORN, R. E., and RUGULA, R. P. 1977. Extrusion: An in-depth look at a versatile process. J. Food Enginering. 49:99-102.

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