A Scanning Electron Microscope Study of Maize Gluten Meal and Soy Coextrudates[1]

P. L. HARRIS, S. L. CUPPETT, and K. W. LEE[2]

ABSTRACT

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The effect of altering extrusion conditions and ingredient levels on the internal structures of maize gluten meal-defatted soy flour coextrudates was studied using scanning electron microscopy. In addition, the relationship of the internal structure to product functionality was examined. Coextrudate water-holding capacity, bulk density, and shear resistance increased as maize protein concentration increased. The dense globular network observed in the low concentration maize gluten meal coextrudates changed to a more organized and striated matrix exhibiting an increased number of aligned, unidirectional fibers. The lacy, thin-walled, single-layered interiors observed in the low-moisture coextrudates were transformed into a multilayered, bundle-like network as moisture levels approached the optimum (32%). Coextrudates processed at high moisture levels exhibited significantly lower BDs ($P < 0.01$) than those processed at lower moistures. As processing temperatures increased, extrudate walls became thinner, increasingly organized, fibrous, and porous. A more striated, organized texture with definite bundle formation was observed in the coextrudates processed at a neutral pH compared with the denser, unorganized interiors observed in coextrudates processed at extreme pH values.

Maize gluten meal (MGM), the proteinaceous residue obtained during the corn wet-milling process, has a protein content of approximately 60%. These zein and glutelin proteins are rich in leucine and glutamic acid but limiting in lysine and tryptophan. Therefore, MGM is usually blended with a legume such as soy to improve its nutritional value. Improvements in the functional and nutritional characteristics of coextruded blends using MGM-defatted soy flour (DSF) and MGM-amaranth were reported by Neumann et al (1984) and Koeppe et al (1987), respectively. After removal of the deleterious pigments and lipids, the resultant maize protein concentrate has a relatively constant water-holding capacity (WHC) before extrusion in comparison to variable values obtained for soy protein concentrate (Sternberg et al 1980).

Extrusion processing variables that can influence product quality include the ingredients and their respective ratios, moisture content of the feed material, pH of the feed material, and processing temperature. Both El-Dash (1981) and Kinsella (1978) have written comprehensive reviews describing the effects of extrusion variables on product functionality and nutritional quality.

The objective of this study was to alter extrusion conditions and examine the microstructures of MGM-DSF coextrudates in relationship to their functionality using scanning electron microscopy.

MATERIALS AND METHODS

Sample Preparation

Wet-milled MGM, containing approximately 70% moisture, was obtained from Pennick-Ford (Cedar Rapids, IA), and DSF was obtained from Archer-Daniels Midland Co., (Decatur, IL). Samples were stored at −20°C before use. Acetone-extracted MGM was prepared for extrusion using the extraction procedure of Harris et al (1987). The acetone extracted MGM and DSF were blended (20:80, 30:70, and 50:50, respectively; w/w, db) and all treatments were taken from these blends. Fifteen treatments were produced per blend ratio that differed in pH, initial moisture content, and extrusion temperature. The range and values of the variables used were established by response surface methodology (Meyers 1976) using a three-variable design (Harris et al 1988). Therefore, pH levels were 2.64, 4.00, 6.00, 8.00, and 9.36, and the initial moisture levels were 13, 20, 30, 40, and 47%. In addition, extrusion temperatures were set at 126, 140, 160, 180, and 194°C.

Each treatment was prepared by slurrying 200 g of the dry blend of MGM-DSF in 800 ml of warm distilled water. Each slurry was blended at high speed in a Waring Blendor to obtain a uniformly smooth mixture (approximately 5 min). All slurries were pH adjusted with 6*N* HCl or 6*N* NaOH to the appropriate pH (Table I). The samples were lyophilized and, 24 hr prior to extrusion, the moisture contents were adjusted to the appropriate level with distilled water (Table I). The blends were transferred to plastic bags and refrigerated.

Extrusion and Functional Analysis

Extrusion was performed as described by Koeppe et al (1987) without the die, in the manner of Feldbrugge et al (1978). Screw speed was 145 rpm. Extrusion temperatures are listed in Table I. WHC was determined in the manner of Neumann et al (1984). Bulk density (BD) was determined as described by Hagan et al (1986). Analyses were performed in triplicate. Lee Kramer shear values, to measure extrudate strength, were determined using the Instron universal testing machine (model 1123, Instron Corp., Canton, MA) with a crosshead speed of 50 mm/min. Force (kg/g) was standardized for the weight of each sample, and shear strength in Pascals was calculated in the manner described by Koeppe et al (1987).

Analysis of variance statistical tests were performed on the functionality data in reference to each extrusion variable for the coextrudates processed from an MGM-DSF blend adjusted to a pH of 4 and 8, with an initial moisture level of 20 and 40% and extruded at 140 and 180°C.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) micrographs were obtained to illustrate differences in microstructures as a variation of ingredient concentration while pH, initial moisture, and extrusion temperature remained constant at 6.00, 13%, and 160°C, respectively. A blend pH of 6.00 was chosen because this level most closely approximated the pH of the unaltered blends (6.38–6.58). Typically, extruded snack-type products require an initial moisture level of 12–15% to ensure sufficient vaporization and textural setting of the final product (Matson 1982). Extrusion temperatures of 120–150°C are recommended (Kinsella 1978). An extrusion temperature of 160°C was chosen because it best fitted this range and the response-surface experimental design. In addition, SEM was used as a tool to illustrate the effects of pH, initial moisture, and extrusion temperature in relationship to functionality for the 50:50 MGM-DSF blend only, because this blend was chosen as optimum based upon additional data outlined by Harris et al (1988). Each extrudate was prepared for SEM examination by using a razor blade to obtain an intact cross section approximately 0.5 cm in height. Samples were mounted on

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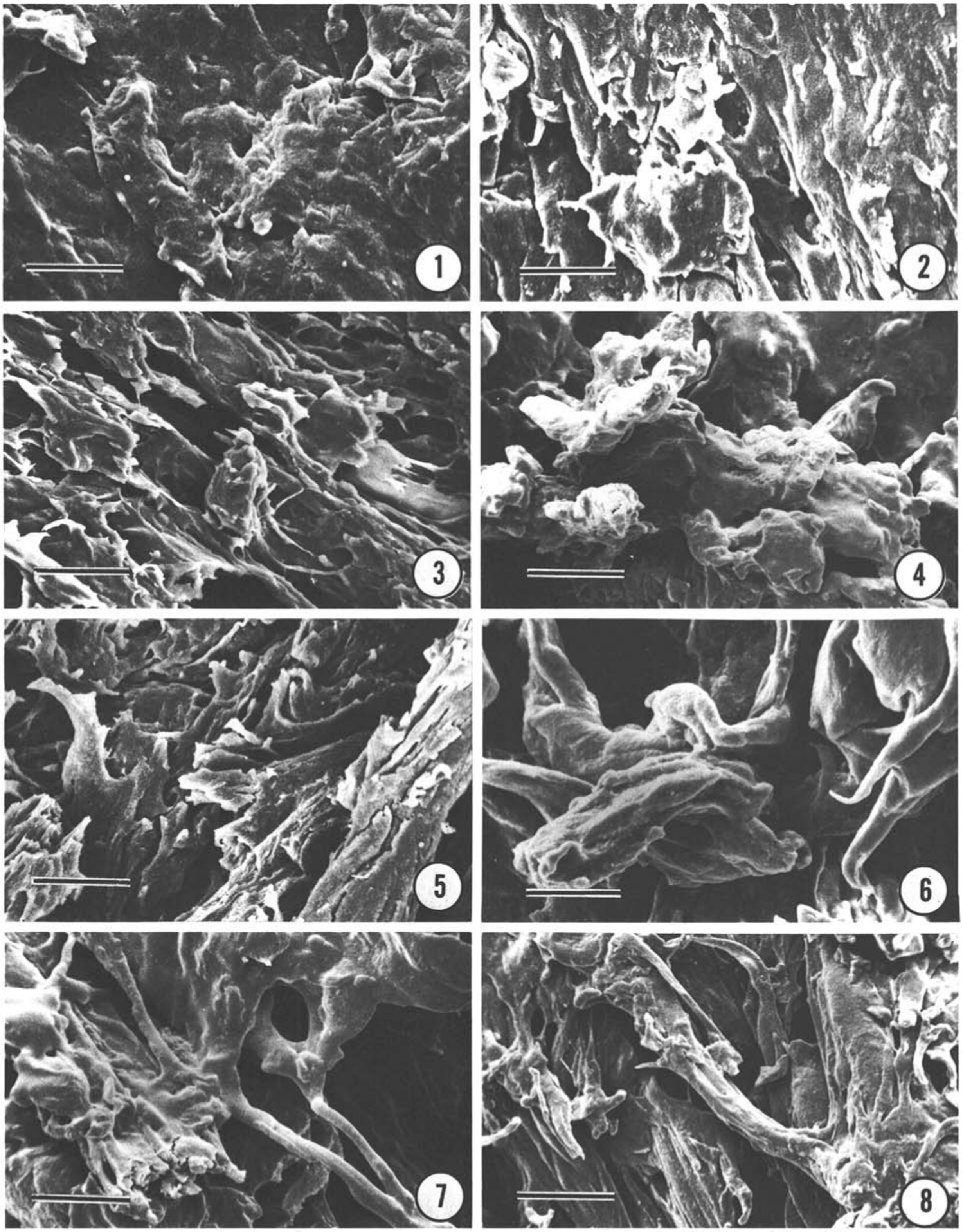
[2]Department of Food Science and Technology, University of Nebraska, Lincoln 68583-0919.

aluminum stubs using copper tape and sputter coated with a 200 Å thick layer of gold using a Technics Hummer II instrument. A Cambridge S4-10 scanning electron microscope with an accelerating voltage of 10 kV was used to view the preparations. Photographs were taken at approximately 1,200 magnification.

RESULTS AND DISCUSSION

Effect of Ingredient Concentration

As the concentration of MGM increased (50%), the dense globular network observed in the low concentration MGM



Figs. 1–8. Scanning electron micrographs of maize gluten meal-defatted soy flour blends coextruded under differing conditions of blend proportions, initial moisture content, initial pH of the blend, and extrusion temperature. Bars = 15 μm (1,200×). **1,** A 20:80 blend, 13% moisture, pH 6.0, extrusion temperature 160° C. **2,** A 30:70 blend, 13% moisture, pH 6.0, extrusion temperature 160° C. **3,** A 50:50 blend, moisture 13%, pH 6.0, extrusion temperature 160° C. **4,** A 50:50 blend, moisture 30%, pH 2.64, extrusion temperature 160° C. **5,** A 50:50 blend, moisture 30%, pH 6.00, extrusion temperature 160° C. **6,** A 50:50 blend, moisture 30%, pH 9.36, extrusion temperature 160° C. **7,** A 50:50 blend, moisture 20%, pH 4.0, extrusion temperature 140° C. **8,** A 50:50 blend, moisture 20%, pH 4.0, extrusion temperature 180° C.

(20–30%) coextrudates (Figs. 1 and 2) changed to a more organized and striated matrix (Fig. 3). This matrix exhibited an increased number of aligned, unidirectional fibers. The progression from a thick, multilayered interior observed in MGM-DSF coextrudates processed with high concentrations of DSF (80 and 70%) to a thinner, single-layered interior observed in coextrudates processed with equal amounts of DSF and MGM is also displayed in these SEM micrographs (Figs. 1–3). An increase in interprotein bonding between the maize and soy components may be responsible for these structural changes. Functionally, as the concentration of MGM in the extrudates increased, lower values for BD, WHC, and shear resistance were observed (Table I). This may occur as a result of the increased concentration of MGM hydrophobic amino acids reducing the protein's ability to interact with water. Functional modifications that accompany protein blending in extrudates have been reported. Bhattacharya et al (1986) reported increases in coextrudate WHC, BD, and shear resistance as soy protein concentration increased in soy protein isolate and MGM blends. Neumann et al (1984) reported lower WHC values for coextruded MGM-DSF blends in comparision to DSF extrudates. The low WHC values exhibited by these coextrudates were increased after autoclaving. Conway and Anderson (1973) evaluated a variety of protein-fortified extruded products and found that the water solubility and water absorption indexes increased and expansion decreased in extrudates as protein content increased to a maximum of 16%. Peri et al (1983), working with coextruded blends containing defatted corn germ flour and milk proteins, reported a symmetrical and inverse relationship between total protein content of the extrudates and their shear resistance. Although the protein content of these extrudates was not determined, theoretically, increasing the percentage of MGM in an MGM-DSF blend would result in an extruded product with a relatively higher protein content.

Effect of pH

The pH of the protein material before extrusion cooking has a significant effect on the fluidity of the dough within the extruder. In addition, final extrudate density, cell size, chewiness, rehydration, and drying characteristics will be modified by the initial pH of the feed material (Kinsella 1978). An alkaline-treated protein will produce a textured product that is less chewy and more water absorbent (Smith 1975).

The micrographs reflect a more striated, organized texture with definite bundle formation in the 50:50 MGM-DSF coextrudates processed with a near neutral pH (Figs. 3, 5, 11, 13, and 14) compared with the denser, unorganized interiors observed in coextrudates processed with more extreme pH values (Figs. 4, 6, 7–10). There was no statistical difference ($P < 0.05$) between the functional properties (WHC, BD, and shear resistance) of the MGM-DSF coextrudates processed from an MGM-DSF blend adjusted to a pH of 4 versus an MGM-DSF blend adjusted to a pH of 8 (Table I). In this study, the 50:50 MGM-DSF coextrudate processed at pH 8, with a moisture level of 40% and an extrusion temperature of 180°C, demonstrated the highest WHC (8.4) (SEM not shown). Smith (1975) observed a direct relationship between BD and shear resistance and an inverse relationship between these two responses and WHC. These findings agree with the observations of Smith (1975), who reported that with acidic conditions, dense extrudates form because of the premature aggregation of the proteins in the extruder barrel. As a result of these circumstances, a greater amount of protein is denatured and becomes insoluble.

The representative 50:50 MGM-DSF (Figs. 7 and 8) coextrudates processed with acidic conditions (pH 4) exhibited a more fibrous nature than the representative alkaline-processed coextrudates (Figs. 9 and 10) at all temperatures and moisture levels. In contrast, the internal structure of the representative alkaline-processed blends (pH 9) resemble dense aggregates of unprocessed dough (Figs. 9 and 10). Smith (1975) reported that the acidification of protein contributes to a lower degree of vaporization as a result of the smaller moisture droplets trapped inside of the extrudate; this produces a denser, chewier extrudate with smaller cell size and a decreased ability to hold water compared with an unacidified protein.

Effect of Initial Moisture Levels

The lacy, thin-walled, single-layered, open interiors observed in the representative lower moisture 50:50 MGM-DSF coextrudates were transformed into a more compact striated network of bundles as moisture levels approached the optimum (32%) (Figs. 3 and 5). At the highest moisture levels (47%), the interior of the 50:50 MGM-DSF coextrudate became denser with some fiber-like formations (Fig. 11). The bulk density values for these MGM-DSF coextrudates were inversely related to the initial moisture level of the blend. MGM-DSF blends extruded with relatively low initial moisture levels (20%) exhibited significantly ($P < 0.01$) greater BD values than those MGM-DSF blends extruded with relatively high initial moisture levels (40%) (Table I). These results agree with Holay and Harper (1982) but disagree with those reported by Bhattacharya et al (1986). Mercier and Feillet (1975) also observed that rehydration of an extrudate is closely related to its density, being markedly more rapid with extrudates of low density. Although there was no significant difference ($P < 0.05$) between WHC and shear resistance values obtained for the MGM-DSF coextrudates processed with initial blend moistures of 20 and 40%, the high (47%) moisture coextrudate (Table I, Fig. 11) exhibited a

TABLE I
Extrusion Variables and Functional Analysis of Maize Gluten Meal-Defatted Soy Flour Coextrudates

Figure	Slurry pH	Initial Moisture (%)	Extrusion Temperature (°C)	Water-Holding Capacity[a]	Bulk Density[a] (g/L)	Strength[b] (10 kPa)
20:80 blend						
1	6.0	13	160	5.7	735	6.6
30:70 blend						
2	6.0	13	160	5.5	621	6.5
50:50 blend						
3	6.0	13	160	6.1	521	4.9
4	2.64	30	160	6.7	500	10.7
5	6.00	30	160	6.7	500	4.7
6	9.36	30	160	5.5	618	3.2
7	4.0	20	140	6.5	694	11.1
8	4.0	20	180	4.6	664	3.1
9	8.0	20	140	3.6	715	6.0
10	8.0	20	180	5.0	744	139.0
11	6.0	47	160	6.0	349	76.4
12	6.0	30	126	5.4	606	13.3
13	6.0	30	194	4.4	564	19.3

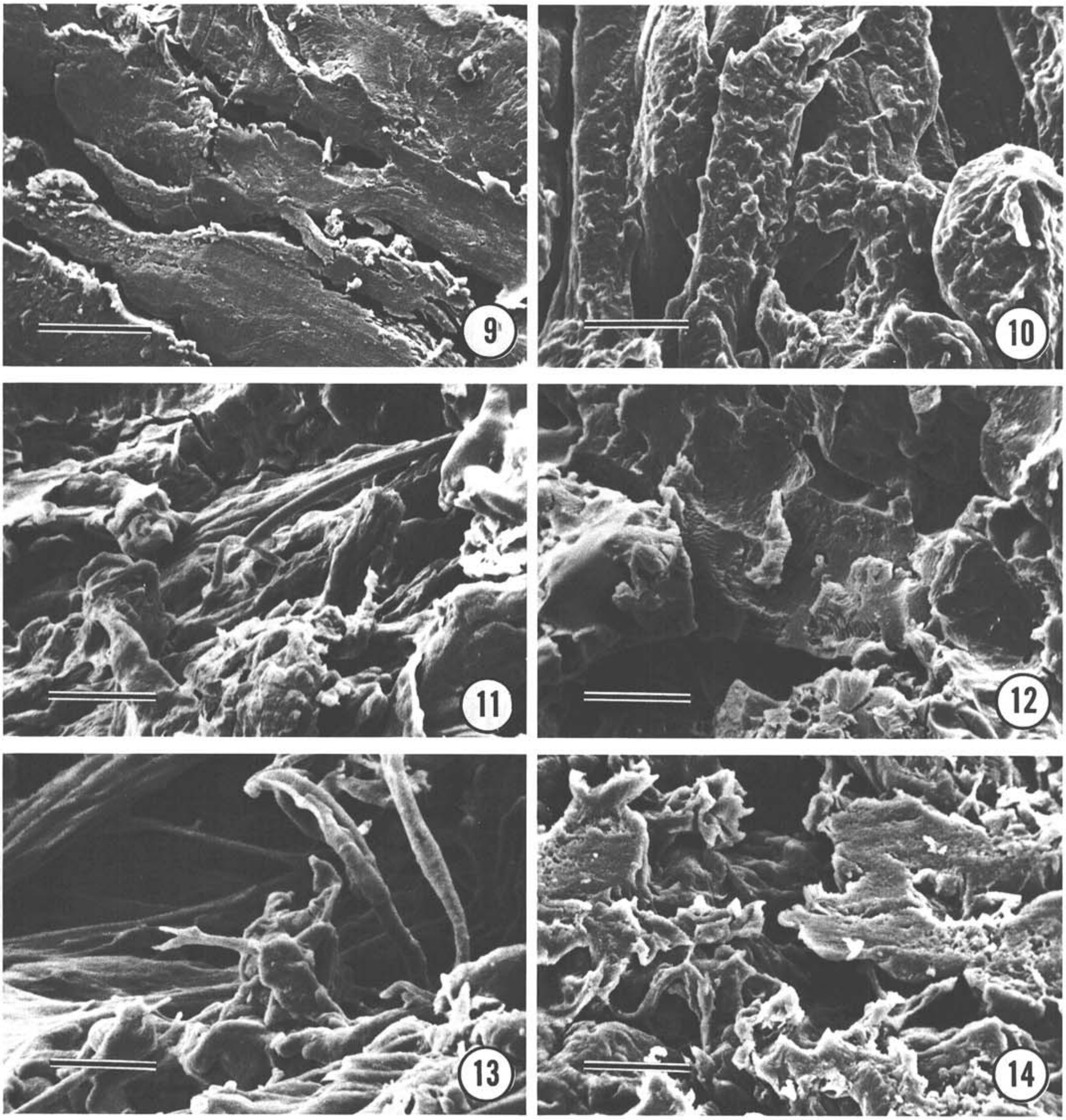
[a] Mean of triplicate analyses.
[b] Strength = Shear resistance (Lee Kramer shear values). Values are means of duplicate analyses.

slightly lower WHC and a much greater shear resistance than the MGM-DSF coextrudate processed at a lower initial moisture level (30%) (Table I, Fig. 5). Koeppe et al (1987) observed a similar relationship between feed moisture levels, WHCs, and shear resistance values with extruded blends of MGM and *Amaranthus hypochondriacus* flour. Conway (1971) reported that if initial blend moisture levels are too high, insufficient vaporization occurs as the product emerges from the extruder. Therefore, extrudates processed at high moisture levels emerge soft and moist and upon drying become hard and tough with possible fissuring (Fig. 11).

Effect of Extrusion Temperature

Extrusion temperatures must be above the vaporization temperature of water (100°C) to permit expansion and flash evaporation. Temperatures of 120 to 150°C are recommended, because extrudates processed at lower temperatures may become easily broken and disintegrate in boiling water (Cummings et al 1972).

At low processing temperatures, the interior of the 50:50 MGM-DSF coextrudate retained the amorphous character of the raw material, resembling uncooked dough (Fig. 12). As processing temperatures increased, extrudate walls became thinner, increasingly organized and exhibited a greater degree of porosity (Fig. 13). There was no statistical difference ($P < 0.05$) between the functional properties (shear resistance, WHC, and BD) of the MGM-DSF coextrudates processed at an extrusion temperature



Figs. 9–14. Scanning electron micrographs of maize gluten meal-defatted soy flour blends (50:50) coextruded under differing conditions of initial moisture content, initial pH, and extrusion temperature. Bars = 15 μm (1,200×). **9,** Moisture 20%, pH 8.0, extrusion temperature 140°C. **10,** Moisture 20%, pH 8.0, extrusion temperature 180°C. **11,** Moisture 47%, pH 6.0, extrusion temperature 160°C. **12,** Moisture 30%, pH 6.0, extrusion temperature 126°C. **13,** Moisture 30%, pH 6.0, extrusion temperature 194°C. **14,** Optimum blend moisture 32%, optimum blend pH 6.25, optimum extrudate temperature 153°C.

TABLE II
Functional Analysis
of Optimum Maize Gluten Meal-Defatted Soy Flour Coextrudates[a]

Sample	Water-Holding Capacity	Bulk Density (g/L)	Strength[b] (10 kPa)
1	2.5	556	57
2	2.5	561	53
3	2.5	576	52
Mean ± SD	2.5 ± 0	564 ± 10.4	54 ± 2.5

[a] Maize gluten meal and defatted soy flour blended in 50:50 ratio (w/w, db). Optimum extrusion parameters: pH 6.25, moisture 32%, extrusion temperature, 153°C, Fig. 14.

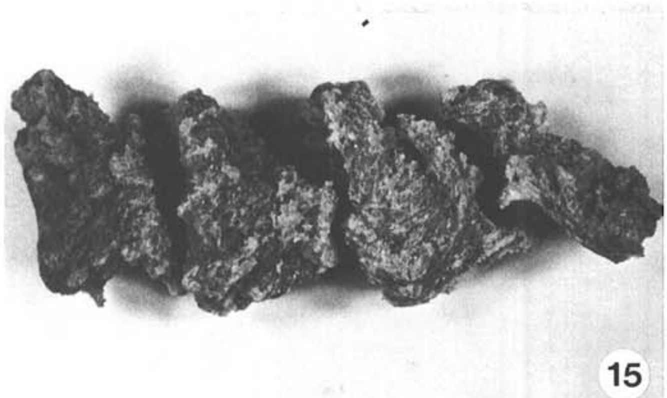


Fig. 15. Exterior view of a maize gluten meal-defatted soy flour blend (50:50) coextruded under optimum conditions (as in Fig. 14): moisture 32%, pH 6.25, extrusion temperature 153°C.

of 140°C versus MGM-DSF coextrudates processed at an extrusion temperature of 180°C. Cummings et al (1972) observed two structural changes as extrusion temperature increased: an increase in aligned fibers with enhanced cohesiveness, and the development of a spongy texture with increased porosity. The results of this study agree with those of Cummings et al (1972), who reported that as the temperature of extrusion increases, the extruded product becomes more resistant to shear and compression but decreases in density. This indicates a greater cohesiveness and increased degree of structural integrity.

At optimum extrusion conditions (pH 6.25, initial moisture 32%, and extrusion temperature 153°C), the interior of the 50:50 MGM-DSF coextrudate takes on a multilayered appearance; the individual layers appear "sponge-like" with the presence of minute pores on the layers (Fig. 14). The bulk density values for these coextrudates averaged 564 g/L (Table II). The ability of these 50:50 MGM-DSF coextrudates to hold water was relatively low (2.5) (Table II). A lower WHC would be desirable in an extruded product that is to be used in a canned pet food where structural integrity and thermostability of the product during retorting would be important. The optimized 50:50 MGM-DSF coextrudate was of intermediate strength because of its relatively high moisture content, but the slightly acidic pH tempered this effect, and the product remained soft, pliable, and chewy upon drying (Table II, Fig. 15).

CONCLUSION

This study represents the first extensive investigation using SEM to illustrate the internal microstructure of MGM-DSF coextrudates in relationship to their functionality. Internal structural fiber formation appeared to be associated with slightly acidic pH in feed materials and elevated extrusion temperatures. Extreme processing conditions, such as low or high pH values in the initial blends, elevated feed material moistures, and low extrusion temperatures resulted in coextrudates with massive, dense, globular interiors. This study demonstrates that SEM can be used as a valuable tool in elucidating the functionality of extruded products in relation to their internal structure.

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