Physical Properties and Some Nutritional Characteristics of an Extrusion Product with Defatted Amaranth Seeds and Defatted Maize Gluten Meal (80:20 Ratio)[1]

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ABSTRACT

Finely ground and defatted seeds of *Amaranthus hypochondriacus* were blended with defatted maize gluten meal in an 80:20 ratio (w/w, db). Samples were adjusted to a moisture content of 12.5% (db) and extruded at a barrel temperature of 150° C with a screw speed of 150 rpm. Analyses of the pre-extrusion blend and extrudates included proximate analysis, Lee-Kramer shear, water holding capacity, bulk density, and puff ratio determinations where applicable. Nutritional considerations included determination of available lysine, trypsin inhibitor activity, in vitro digestibilities, amino acid analysis, and calculated protein efficiency ratios. Background levels of trypsin inhibitor were essentially destroyed with extrusion cooking, whereas no loss in lysine availability was evident. An exceptionally high in vitro digestibility (85.4%) was determined for the extruded product in addition to a calculated protein efficiency ratio value of 1.27.

Maize gluten meal (MGM), the proteinaceous by-product of the wet-milling process, is used extensively in poultry feed as a valuable xanthophyll source (Reiners et al 1973) and has been advocated as a partial flour replacement for bakery products and pasta (Feldberg 1965). However, before MGM can be used as a human food, its functionality and stability must be improved by extracting the lipids, pigments and undesirable odors and flavors imparted by bisulfites during processing. The resultant maize protein concentrate (MPC) is high in protein (60%), rich in leucine and glutamic acid but limiting in lysine and tryptophan (Sternberg et al 1980). The successful coextrusion of MPC-defatted soy flour blends to obtain a uniquely textured protein product (Neumann et al 1984) may provide a practical method for improving MPC's functional and nutritional attributes.

The amaranths are broad-leafed plants that have attracted renewed interest as a potential food source because they grow vigorously, resisting drought, heat, and pests, and they produce a significant amount of edible cereal-like grain that contains relatively high contents of protein, lysine, and calcium in comparison to the true cereal grains such as wheat (Pant 1983). Research on the effects of processing whole grain amaranth or compositional fractions appears to be limited to studies related to toasting and popping (Betschart et al 1981, Oke 1983) and the incorporation of amaranth flour into cereal-based products such as bread (Saunders and Becker 1984) and tortillas (Sanchez-Marroquin 1980). Amaranth's high lysine content has been used to supplement wheat (Pant 1983), rice and red gram bean (Subramanian and Srinivasan 1951, Kundaji and Radha Krishna

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Rao 1954), corn and sorghum (C. Klopfenstein 1986, *personal communication*).

Extrusion cooking can improve ingredient utilization, reduce energy costs, and produce a nutritionally balanced product in an appetizing form. Kinsella (1978) reviewed extrusion variables and their effects on functional characteristics in different food systems. Temperatures at extrusion must be above the vaporization temperature of water (100° C) to permit product expansion and flash evaporation. Temperatures of 120–150° C are recommended (Cummings et al 1972, Mercier and Feillet 1975). Cummings et al (1972) also observed a decrease in product density with increasing processing temperatures. Peri et al (1983), working with an extruded corn germ-milk snack product, reported that increasing the protein concentration in an extruded product decreases its expansion. Decreasing the gluten level or increasing the lipid content of the starting material will weaken or tenderize the extrudate. Smith (1975) concluded that the pH of the protein material before extrusion cooking has a significant effect on the textural and functional characteristics of the final product. Compared to an unmodified protein, acidification of the protein will result in a more chewy textured product, smaller cell size, lowered density, and a decreased ability to absorb water. An alkaline-treated protein will produce a textured product that is less chewy and more water absorbent. Protein quality, determined by net protein ratio and net protein utilization values, improved with extrusion processing (Srihara and Alexander 1984).

Extrusion cooking of amaranth, alone or in combination with nutritionally complementary cereal grains such as maize, has not been reported. The objectives of this study were to coextrude amaranth and MGM and to determine several of its functional and nutritional characteristics.

MATERIALS AND METHODS

Enzymes

Bovine trypsin (lot 24F-8011), protease (type XIV, lot 120F-0589), peptidase (lot 119C-8011), α-chymotrypsin (type II, lot 92F-8205), hog pancreatic trypsin (type IX, lot 72F-0356), *N*-α-benzoyl-DL-arginine-*p*-nitroanalide, and casein were obtained from Sigma Chemical Co. (St. Louis, MO).

Sample Preparation

Amaranthus hypochondriacus grain was obtained from Post Rock Natural Grains (Luray, KS). Wet MGM was obtained from Pennick and Ford (Cedar Rapids, IA). Samples were stored at −20° C until used. The seeds were finely ground using a Janke and Kunkel Ika-Werk grinder (type A10S1, Staufen i, Breisgau, FRG) and lipids were removed using a 4-hr hexane extraction (AOAC 1980, method 14.018). Wet MGM, containing approximately 70% moisture, was lyophilized at 30° C before extracting for 4 hr with histological grade acetone (AOAC method 14.018, 1980). Amaranth (AM) and MGM were blended in an 80:20 ratio (w/w, db). Residual solvent was removed by sample suspension in warm, distilled water followed by centrifugation at 5,000 rpm for 10 min; washing was repeated four times. The samples were air-dried, and moisture contents were adjusted to 12.5% and refrigerated 24 hr prior to extrusion. Four replicate samples were prepared.

Extrusion

Extrusion was performed using a C. W. Brabender extruder (model 2802) equipped with a 19-mm diameter single screw having a 20:1 length-to-diameter ratio and a 3:1 compression ratio. Extrusion parameters were 150° C barrel temperature, a screw speed of 150 rpm, and a 3-mm restriction die or nozzle. Four replicate samples were extruded. Yellow corn meal was extruded as a control using the same parameters.

Proximate Analysis

Proximate analysis of the extrudates included crude protein (Kjeldahl; AACC method 46-10, 1983), crude fiber (AOAC method 14.020), moisture (AACC method 44-19), ash (AACC method 08-01), lipid (AOAC method 14.018), and carbohydrate by difference. Triplicate determinations were measured on the four replicate samples.

Functional Evaluation

Diameters of the extrudates were measured and puff ratios calculated as:

$$\frac{(\text{extrudate diameter})^2}{(\text{die diameter})^2}$$

Six measurements were taken on each segment of three replicate samples.

Lee-Kramer shear values were determined using the Instron universal testing machine model 1123 (Instron Corp., Canton, MA) using a cross-head speed of 50 mm/min. Three replicate samples measuring 67 mm in length were evaluated in triplicate. Shear strength in Pascals was calculated as:

$$\frac{\text{shear force (kg)} \times 9.81\ \text{m}^2/\text{s}}{\text{area of extrudate (m}^2) \times N}$$

where N = number of shear surfaces.

Water-holding capacity (WHC) was determined in the manner of Neumann et al (1984). Samples were measured in triplicate.

Bulk density was determined in the manner of Hagan et al (1986) at ambient temperature (24° C). Samples were measured in triplicate.

Extrudate microstructure was observed using scanning electron microscopy. A razor blade was used to obtain an intact cross section of the extrudate approximately 0.5 cm in height. Samples were mounted on aluminum stubs using silver paste and then coated in a Denton vacuum evaporator model DD502 with a thin layer of carbon followed by a 300–400 μm thick layer of gold. An Amray scanning electron microscope (model AMR1000A) with an accelerating voltage of 20 kV and a zero stage tilt was used to view the preparations. Photographs were taken at 20× and 50× magnification.

Nutritional Evaluation

Available lysine was analyzed as outlined by Hurrell et al (1979). Samples were measured in triplicate. Trypsin inhibitor activity was determined using the method of Kakade et al (1974). Measurements were performed in triplicate for each of three samples. One trypsin inhibitor unit (TIU) was defined as the amount of inhibitor that caused 10% inhibition of trypsin in 10 min under the described assay conditions.

In vitro digestiblity was determined according to AOAC method 43.C16, 1980. Single determinations were performed on four replicate samples. Amino acid analysis was performed using a Beckman 120C amino acid analyzer equipped with an automatic sample applicator and a system AA computing integrator. Calculation of amino acids was based on an experimentally determined nitrogen factor of 5.85 for amaranth (Becker et al 1981). Duplicate determinations were conducted on duplicate samples. Calculated protein efficiency ratios (CPERs) were derived from these data as described in AOAC methods 43.C10–43.C18.

RESULTS AND DISCUSSION

A moisture level of 12.5%, pH 6.0, 80:20 AM-MGM blend, and extrusion parameters were selected from preliminary experimental data to achieve the desired product. As reported by Conway and Anderson (1973), lipid content decreases product expansion. To achieve maximum expansion, the lipids in both the AM and MGM were removed by hexane and acetone extraction, respectively, which was shown in preliminary studies to achieve maximum lipid, pigment, and odor removal.

Nutritional Evaluation

A comparative proximate analysis of the AM-MPC extrudates, AM, and MGM are presented in Table I. Lipid levels decreased to

<0.1% in the samples after hexane and acetone extractions of the AM and MGM, respectively. These results are comparable to the ethyl acetate procedure described by Sternberg et al (1980) for use with MGM.

By using an initial moisture level of 12.5% in the unextruded blend, the final moisture level of the extrudates averaged 7.7% (db), which was within the range (4–8%) reported by Matson (1982) as characteristic for an extruded snack product. The moisture concentration in the pre-extruded mix markedly affects product texture. If moisture is too high, the product does not expand because of insufficient water vaporization. This results in a soft, moist extrudate that may toughen and become hard upon storage (Conway 1971).

Although a definite browning of the extrudates was observed, preliminary studies in this laboratory did not show an increase in reducing sugars due to starch hydrolysis. Extrusion processing of the AM-MPC blends did not result in a decrease in lysine availability (Table II). Available lysine values of the extrudates ranged from 2.82 to 2.96 g of lysine/100 g protein and were comparable to that of the unextruded blend and the total lysine value (Table III). These values were lower than those expected based on reported lysine values for amaranth (5.0 g/100 g protein) and MGM (1.7 g/100 g protein) reported by Cole (1979) and Shroder and Heiman (1979), respectively, but agree with Zeece (*personal communication*) who observed no significant decrease in lysine availability with extrusion cooking of MGM-corn germ blends. DeMuelenaere and Buzzard (1969) observed no significant decrease in lysine availability with extrusion cooking of full-fat soybeans but a 28% decrease in available lysine in extruded products containing skimmed milk. Bjorck and Asp (1984) observed 63–100% lysine retention in extruded wheat flour and concluded that lysine retention is affected positively by an increase in feed rate and negatively by an increase in screw speed.

The initial level of trypsin inhibitor (TI) in the AM-MPC blend (0.52 TIU/mg, Table II) compared well with levels reported for crude extracts of *A. hypochondriacus* (Koeppe et al 1985) but were slightly greater than noted for *A. cruentus* (Saunders and Becker 1984). Extrusion processing reduced the level of TI in the AM-MGM extrudates to an average of 0.20 TIU/mg (Table II). Levels of TI, before and after extrusion, were relatively small and probably not nutritionally significant. The observed loss of TI activity with extrusion is in agreement with Mustakas et al (1970), who reported that the short time needed for extrusion cooking adequately inactivated growth inhibitors such as TI in full-fat soy flour.

In vitro digestibility values increased slightly from 82.1% in the unextruded blend to an average of 85.4% in the AM-MGM extrudates (Table II). This was as expected because of the denaturation of protein and the inactivation of antinutritional factors caused by the elevated temperatures involved in extrusion cooking. These results are similar to those obtained by Neumann et al (1984), who reported an increase in digestibility with soy-MGM extrudates.

A CPER value of 1.27 (Table IV) was determined for the AM-MPC extrudates from the in vitro digestibility values and amino acid profile presented in Tables II and III. The calculated CPER value was as expected when estimated from the reported amino

TABLE I
Proximate Analysis[a] of Maize Gluten Meal (MGM), Amaranth (AM), and the 80:20 AM-MGM Extrudates[b]

Component	MGM[c]	AM[c]	AM-MGM Extrudates (1)	(2)	(3)	(4)	$\bar{x}$	SD
Crude protein (N×5.85)	62.0	15.9	22.9	23.5	23.1	24.1	23.4	0.5
Moisture	6.0	7.0	7.8	7.8	7.6	7.8	7.8	0.1
Crude fiber	5.0	5.0	2.8	2.8	2.8	3.2	2.9	0.2
Ash	3.0	3.7	2.3	2.7	2.4	2.6	2.5	0.2
Lipid	3.0	6.6	0.3	0.1	0.3	0.1	0.2	0.01
Carbohydrate	22.0	62.0	63.9	63.1	63.8	62.2	63.3	1.03

[a]%, Wet basis.
[b]Triplicate determinations on each sample.
[c]Unextracted.
[d]By calculated difference.

TABLE II
Nutritional Evaluation of the 80:20 Amaranth-Maize Gluten Meal Extrudates

Sample	Available Lysine[a] (g Lys/100 g Pro)	Trypsin[a] (TIU[b]/g Pro)	In Vitro Digestibility[c] (%)
80:20 AM-MPC blend[d]	2.82	0.52	82.1
80:20 AM-MPC extrudates			
1	2.88	0.20	84.6
2	2.82	0.24	85.9
3	2.91	0.18	85.0
4	2.96	0.19	85.7
Extrudates ($\bar{x}$ ± SD)	2.89 ± 0.06	0.20 ± 0.03	85.4 ± 0.6

[a]Triplicate determinations on four replicate samples and unextruded blend. Pro = Proline.
[b]One trypsin inhibitor unit (TIU) was defined as the amount of inhibitor which caused 10% inhibition of trypsin in 10 min under the described assay conditions.
[c]Single determinations on four replicate samples and unextruded blend.
[d]Unextruded; MPC = maize protein concentrate.

TABLE III
Amino Acid Analysis of Amaranth, Maize Gluten Meal, and the 80:20 Amaranth-Maize Gluten Meal (AM-MGM) Extrudates[a]

Amino Acid	MGM[b]	Amaranth[c] (g/16 g N)	AM-MGM Extrudates
Alanine	5.2	···	5.80
Arginine	1.9	···	5.82
Aspartic acid	3.6	···	7.95
Cysteine	1.1 (Met+Cys)	4.7	1.98
Glutamic acid	13.8	···	18.62
Glycine	1.6	7.4	5.19
Histidine	1.2	2.5	2.39
Isoleucine	2.3	3.9	4.04
Leucine	10.1	5.7	12.06
Lysine	1.0	5.5	3.19
Methionine	1.9	···	1.85
Phenylalanine	3.8	4.0	5.95
Proline	5.5	···	7.08
Serine	3.1	6.3	6.12
Threonine	2.0	3.6	3.82
Tryptophan	0.3	···	1.13
Tyrosine	2.9	3.3	3.76
Valine	2.7	4.5	4.54

[a]Duplicate determinations on two replicate samples.
[b]Shroder and Heiman 1979.
[c]*Amaranthus hypochondriacus*, Carlsson 1982.

TABLE IV
Calculated Protein Efficiency Ratio[a] (CPER) of the 80:20 Amaranth-Maize Gluten Meal Extrudate

Amino Acid	Sample g/16 g N	Sample % FAO[b]	Casein Standard g/16 g N	Casein Standard % FAO[b]
Isoleucine	4.04	86	5.24	117
Leucine	12.06	146	9.64	122
Lysine	3.19	50	8.21	135
Methionine + Cysteine	3.83	93	3.54	90
Phenylalanine + Tyrosine	9.71	136	10.38	153
Threonine	3.82	82	4.13	92
Tryptophan	1.13	101	1.09	102
Valine	4.54	78	6.59	119
CPER = 1.27				

[a]Assuming casein = 2.50 PER and using Food and Agricultural Organization standard profile and a digestibility of 85.4% for the sample and 89.4% for the casein.
[b]Percentage of Food and Agricultural Organization (FAO) standards for protein requirements (FAO/WHO 1973).

acid and digestibility values for AM (Cole 1979) and MGM (Shroder and Heiman 1979) (Table III). Based upon lysine values for MGM and AM, an estimated lysine value for an 80:20 AM-MGM blend would be 3.3. Even though by weight the blend is 80:20 in favor of AM, on a protein basis AM and MGM contribute equally because of MGM's much higher protein content (62 vs. 16%). Acton et al (1983) observed a decrease in CPER and PER values with extrusion cooking of fully defatted peanut flakes, and Neumann et al (1984) observed lower CPER values for MGM-soy extrudates than did Satterlee et al (1982) for textured soy protein. Conversely, the protein quality of five blends of plant protein sources (using combinations of corn, wheat, rice, oats, white beans, and fat-extracted meals of soybean, peanut, and sesame) were reported to increase by Srihara et al (1984), and improvements in PERs also were reported by Bressani et al (1978).

Functional Evaluation

A summary of the functional properties of the AM-MGM extrudates is shown in Table V. WHC values increased slightly as compared with the unextruded blend. The expansion ratio was approximately 2.5 times greater than the yellow corn meal standard (Conway and Anderson 1973) and 3.5 times greater than that of the yellow corn meal control extrudate used in this study. Conway and Anderson (1973) observed that gluten protein increases expansion when added to corn snack meal. The high level of protein in the AM-MGM blend may be partially responsible for the elevated expansion ratios observed in these extrudates. Preliminary studies indicate that expansion ratios increased and WHC decreased with decreasing moisture levels. Expansion ratios increased with increasing screw speeds. These effects are well documented in a review by El-Dash (1981).

Bulk density values of the AM-MGM extrudates (461–490 g/L) were less than that of the yellow corn meal control extrudate (Table V). Ayres et al (1974) and Hagan et al (1986) reported a decrease in bulk density with a corresponding increase in expansion ratio for extruded peanut flour.

The shear strength of the extrudates ranged from 521 to 657 kPa (Table V). Preliminary studies indicated that extrudate strength was increased by increasing moisture levels but decreased by increasing screw speeds. Cummings et al (1972) observed an increase in extrudate shear strength as processing temperatures increased from 120 to 150° C but a rapid decrease above 160° C. At higher temperatures, excessive fissuring occurs that weakens the cohesive strength of the extrudate.

Dried AM-MGM extrudates contained a greater number of individual cells that were more compact, of uniform size, and had a thinner wall structure with more convolutions than the corn meal control. The thinner walls and smaller air cell diameters of the AM-MPC extrudates may account for the increased expansion and lower bulk density of these extrudates when compared to the corn meal extrudate (Fig. 1). Although a thicker wall structure is usually associated with a greater shear force (Hagan et al 1986), the compact, multicellular, convoluted structure of the AM-MPC extrudates may contribute to the high shear force observed.

TABLE V
Functional Evaluation of 80:20 Amaranth-Maize Gluten Meal Extrudates

Sample[a]	Puff Ratio[b]	Strength[c] (kPa)	Bulk Density (g/L)	WHC[d]
80:20 AM-MPC blend[e]	...	...	...	3.4
80:20 AM-MPC extrudate				
1	6.8	657	464	4.7
2	6.4	622	461	4.5
3	6.9	521	490	4.5
Extrudates ($\bar{x} \pm$ SD)	6.7 ± .26	600 ± .71	472 ± .16	4.6 ± .12

[a] MPC = Maize protein concentrate.
[b] Puff ratio defined as: (extrudate diameter)^2/(die diameter)^2. Nine determinations on three replicate samples.
[c] Strength calculated as: [shear force (kg) × 9.81 m^2/s]/[area of extrudate (m^2) × N]. Duplicate determinations on three replicate samples.
[d] WHC = Water holding capacity. Triplicate determinations on three replicate samples.
[e] Unextruded.

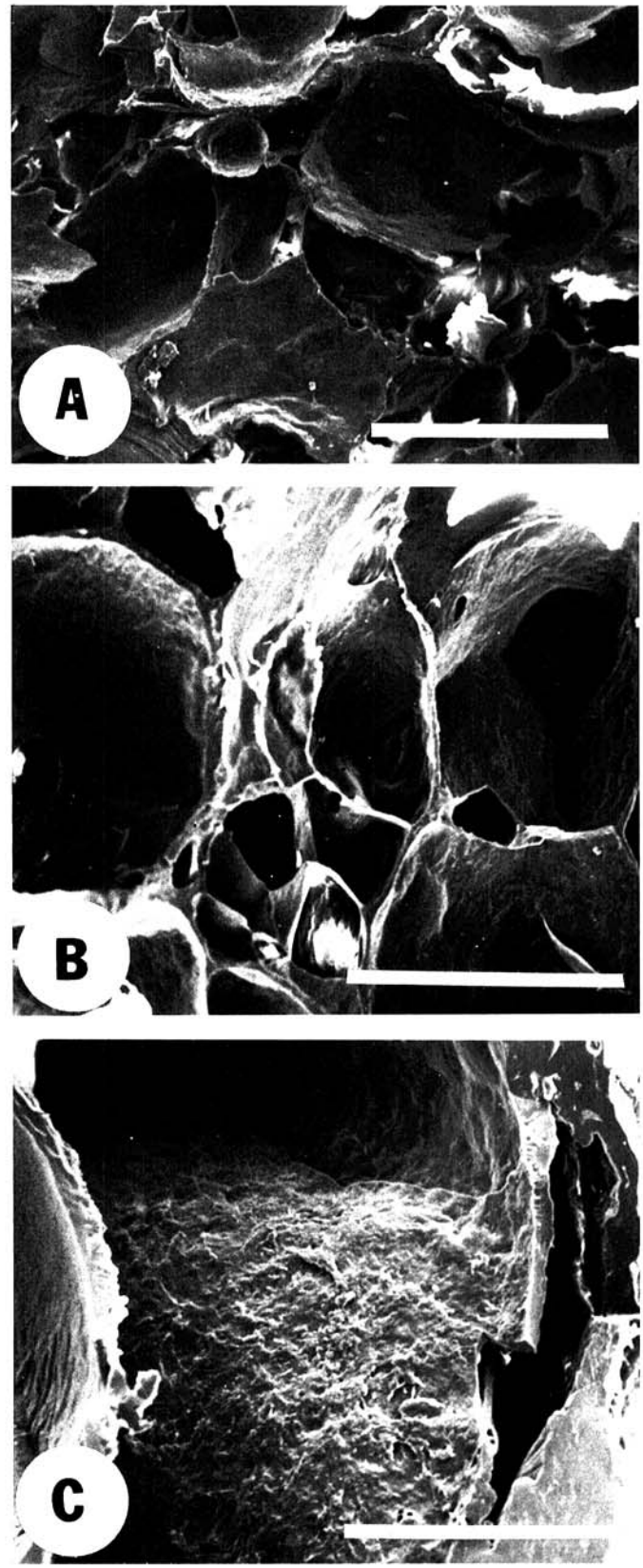

Fig. 1. Scanning electron micrographs of amaranth/maize gluten meal extrudate cross-sections at **A,** 20×, bar = 1,000 μm, **B,** 50×, and **C,** a corn meal extrudate for comparison at 50×, bars = 500 μm.

CONCLUSION

The coextrusion of AM and MGM produced a puffed product having a crude protein content of 24% that was of relatively high quality. AM and MGM are nutritional complements, as AM is first limiting in leucine but high in lysine, and MGM is low in lysine but high in leucine. This complementarity appears to be optimum at the 80:20 ratio employed, although CPER values cannot be relied upon to give an accurate evaluation of nontypical cereal grains such as amaranth. Therefore, the true biological value of the proteins in the extruded product as well as digestible calories must be assessed. In addition, organoleptic characteristics, such as odor, flavor, and mouthfeel must be evaluated to determine the feasibility of this extruded product as a possible human food source. The results of this study supported the hypothesis that potential food resources exist in areas of industrial by-products such as MGM, as well as in underutilized cereal-like grains such as amaranth.

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