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Protein digestibility of extruded cereal grains

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Seven whole-grain cereals were examined to determine extrusion processing effects on in-vitro protein digestibility. Eight extrusion conditions were applied and effects of cereal variety, extrusion temperature, RPM and feed moisture on in-vitro protein digestibility were studied. Protein digestive acceptability of unprocessed and extruded cereals was determined using an in-vitro method of enzymatic hydrolysis. The most protein-digestible products were produced at extrusion combination: 15% feed moisture, 100°C/150°C product temperature and 100 rpm. This effect was significant when considered across all cereals. Results of this study suggest that a particular combination of extrusion process conditions may be applied when extruding a wide variety of cereals, with the benefit of improving protein digestibility.

INTRODUCTION

Seventy percent of protein available on a worldwide scale for human consumption is derived from plant sources with cereal grains contributing 50% of total proteins consumed (Chung & Pomeranz, 1985). Based on the widespread usage of cereals as a major food source for humans and animals, it is a concern to maintain or even improve protein quality of cereal grains during processing.

It is the amino acid composition, bioavailability and digestibility of a protein that defines its nutritional quality (Pacquet, 1987; Finley, 1989; Phillips, 1989). Lysine is the limiting amino acid in all cereals and the essential amino acids isoleucine, threonine and tryptophan may also be present in inadequate quantities. Traditionally, protein quality has been improved in cereal grains by amino acid supplementation, introduction of new, superior protein varieties or development of low-cost food blends complementary in amino acid profile (Mertz, 1978). Processing methods, such as extrusion, may also provide a means of favourably altering protein structure in such a way that renders food protein more digestible (MacLean *et al.*, 1983; Mertz *et al.*, 1984; Coulter & Lorenz, 1991), thus improving one facet of that which defines protein nutritional quality.

Enzymatic assays are one means of measuring protein digestibility. In-vitro, enzymatic determination of protein digestibility has been observed to correlate closely with in-vivo studies (Hsu *et al.*, 1977; Holm *et al.*, 1985; Lee *et al.*, 1985). Although in-vitro systems do not compare to in-vivo systems with regard to physiological complexity, they provide models for examining characteristics of foods and processing conditions that may affect digestibility and possibly rates of absorption (Snow & O'Dea, 1981; Lee *et al.*, 1985). In-vitro methods are also less expensive and time consuming than methods utilizing humans or animals and provide a means of routine analysis for many food materials. Estimates of in-vitro digestibility in conjunction with other nutrient composition data have potential use in the evaluation of grain nutritional value early in the breeding cycle as well as providing useful information for future labelling purposes.

Accordingly, this investigation was undertaken to study the effects of grain type and extrusion process conditions on in-vitro protein digestibility, as well as to determine optimum extrusion process conditions required for improving protein digestibility characteristics in a wide variety of whole-grain cereals.

MATERIALS AND METHODS

Sample identification

Rye, winter wheat, quinoa, corn and millets were obtained from the Colorado State University Agronomy Department. Low tannin sorghum and sorghum-containing tannins were obtained from Texas A & M University, College Station, Texas.

Cultivar types, growing season and location of where each grain was grown are as follows. *Rye*: cultivar was Maton, grown at the Irrigated Desert Research Station, Brawley, California on a Holtville silty clay soil; *winter wheat*: a composite was used which was obtained from the winter wheat nursery, grown during the 1989

growing season in Fort Collins, Colorado; *quinoa* (*Chenopodium quinoa* Willd): currently the only available variety of quinoa in the United States. Cultivar used was Colorado D407, a Chilean landrace, grown during the 1987 growing season in experimental plots located in the San Luis Valley of Colorado; *corn*: cultivar used was CC-136, grown during the 1988 growing season in Fort Collins, Colorado; *millets*: cultivar used was Colorado-135, grown during the 1989 growing season in Fort Collins, Colorado; *low tannin sorghum*: cultivar used was a white food grade sorghum (Dorado), grown during the 1989 growing season in College Station, Texas; *high tannin sorghum*: cultivar used was brown, ATX 623 × SC103-12E, also grown during the 1989 growing season in College Station, Texas.

Sample preparation

Each whole-grain was milled prior to extrusion through a 2 mm mesh screen using a Thomas-Wiley Laboratory Mill (model 4). Prior to conducting extrusion-processing, the moisture content of each cereal was determined according to AACC methodology (AACC, 1983). Each analysis was done in triplicate. Following initial moisture determination, two 1000 g samples of each grain were weighed out. One 1000 g sample was adjusted to 15% moisture, the second 1000 g sample was adjusted to 25% moisture. Moisture additions were carried out by adding the appropriate amount of 20°C tap water by pipet to the grains as they were mixed (20 min, low speed), in a Hobart mixer, model a-120 (Hobart Manufacturing Company, Troy, OH). The mixtures were allowed to equilibrate for 48 h at room temperature in air-tight plastic bags. This process was repeated for each grain type to provide duplicate, moisture-adjusted samples.

Saponin removal

In order to remove saponins present in quinoa samples, quinoa was mechanically abraded using a barley pearling machine modified for on-farm use. The mechanics of this process uses gravity to move quinoa through a hopper forcing it into a tapered cylindrical chamber. This horizontal chamber has a stationery, perforated metal cone on the outside with a tapered revolving carborundum stone on the inside. The quinoa is forced under pressure against itself within the cone. The pericarp, as it is rubbed off, is forced out through small perforated holes in the outside metal cone.

Extrusion

A single screw Brabender Plasticorder Extruder, model PL-V500 (C. W. Brabender Instruments, Inc., South Hackensack, NJ) with a 19·05 mm barrel diameter, a 20 : 1 length to diameter ratio and eight 0·79 × 3·18 mm longitudinal grooves, was used. The extruder consisted of two electrically-heated zones (zone 1/zone 2). Desired product temperature was maintained by thermostats while compressed air-cooled collars around the barrel improved temperature control. Thermocouples were present to monitor product temperature. These contacted the product at the inside barrel wall surface. Each sample was extruded at two product temperatures: 80°C/100°C and 100°C/150°C at the feed and compression sections, respectively.

The extruder was equipped with a variable speed drive allowing all samples to be run at two screw speeds: 100 and 150 rpm. A 4·76 mm die diameter and a 3 : 1 screw compression ratio was used on all trials. All samples were run in duplicate.

Proximate analysis

Prior to analysis, all samples were ground through a 1 mm mesh screen using a UDY Cyclone sample mill (UDY Corp., Ft Collins, CO). Proximate analyses were performed on unprocessed samples of each cereal (Table 1). All analyses were performed in duplicate. Moisture, crude fat, and ash were determined according to AACC procedures (AACC, 1983). Protein was determined on 0·5 g samples by the micro-Kjeldahl method with the boric acid modification (AACC, 1983). The nitrogen conversion factor used was 6·25 for all samples except wheat, which was calculated using 5·7. Neutral Detergent Fibre was determined by the method described by Van Soest (1963).

In-vitro protein digestibility studies

To ensure particle size uniformity for in-vitro digestibility studies, all extruded and non-extruded samples prior-milled to 1·00 mm mesh, were passed through two USA Standard Testing Sieves (W. S. Tyler, Inc., Mentor, OH), mesh size #80 and #40, to determine approximate grain particle size. From a representative sample, it was determined that approximately 70% of all grain particles used for digestibility trials were between 180 and 420 μm in size.

In-vitro nitrogen digestibility was determined using a modification of the method described by Maga *et al.* (1973). Based on protein content, samples were

Table 1. Proximate composition of unprocessed cereal grains (% dry basis)

Cereal	Ash	Fat	Nitrogen	Protein	NDF[a]
Sorghum (high tannin)	2·09	3·69	2·00	12·49	7·3
Sorghum (low tannin)	1·48	3·11	1·86	11·64	6·4
Millet	4·02	4·12	1·97	12·32	13·5
Quinoa	3·53	4·88	2·77	17·34	5·5
Wheat	1·88	1·10	2·83	16·13	9·7
Rye	2·45	1·83	2·57	16·06	8·6
Corn	1·36	3·80	1·75	10·92	10·2

Protein = % nitrogen × 6·25 (% nitrogen × 5·7, for wheat).
[a] NDF = Neutral Detergent Fibre.

measured so as to contain 2 mg nitrogen per millilitre. The samples were suspended in 40 ml distilled water and allowed to rehydrate for 60 min at 5°C with intermittent stirring.

After rehydration, the samples were placed in a 37°C water bath and the pH was adjusted to 7 using 0·01, 0·1 and 1·0 N HCl and NaOH solutions. A digital Mini-pH Meter (VWR Scientific, Inc., San Francisco, CA) calibrated using pH buffer 7 at 37°C was used to monitor the pH. Three millilitres of lyophilized, crystallized trypsin (Sigma Chemical Co., St Louis, MO), at a concentration of 40 mg/ml, was added to each sample. The trypsin had an activity of 13 766 BAEE units/mg protein. Changes in pH were measured at one minute intervals for ten minutes. Each analysis was performed in duplicate.

Experimental design and statistical analysis

The experimental design was a split-split-plot arranged in block fashion with extruder barrel temperature functioning as the main plot, screw speed as the split-plot and feed moisture as the split-split-plot. Each processing variable was run using two levels. Product temperatures were 80°C/100°C and 100°C/150°C at the feed and compression sections, respectively. The two levels for screw speed were 100 and 150 rpm and for feed moisture, 15 and 25%. This resulted in eight extrusion process conditions. A 4·76 mm die diameter and 3 : 1 screw compression ratio were used for all samples extruded.

The data were analyzed using the Statistical Analysis System (SAS) program General Linear Models Procedure (SAS Institute, Inc., 1987). When the split-plot and split-split-plot error terms were not different, they were pooled. Actual mean comparisons were performed using Least Significant Differences (LSD) to determine significance of cereal variety, extrusion temperature, rpm and feed moisture on in-vitro protein digestibility.

RESULTS AND DISCUSSION

In-vitro protein digestibility

Assessment of in-vitro protein digestibility by monitoring change in pH after cereal–trypsin incubation is a model simulating what occurs during digestion *in vivo*.

The trend seen during in-vitro proteolysis of cereal grains over time with trypsin enzyme is shown in Fig. 1. During cereal–enzyme incubation, hydrolysis occurs and amino acids are released from the peptide chain. This results in decreased pH, a marker for increased protein digestibility.

Maga *et al.* (1973) noted trends similar to that shown in Fig. 1 with steam cooking. It was concluded that heating may benefit protein digestibility by rendering the protein more susceptible to hydrolysis due to structural changes, destruction of anti-enzymatic factors or decrease lipid–protein, starch–protein complexes.

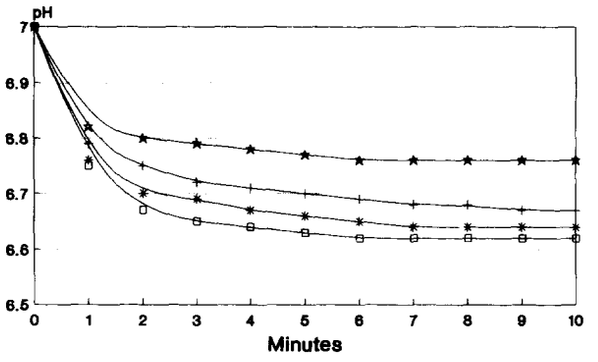

Fig. 1. Decreased pH observed over time during cereal–trypsin incubation. —☆— = Corn; —+— = quinoa; —•— = high tannin sorghum; —□— = wheat.

As shown in Fig. 1, the pH drop is rather sudden initially, and then becomes quite linear over the last four minutes of incubation as substrate to enzyme ratio declines. pH values from the last four minutes of incubation (data not shown) were, therefore, averaged to provide an overall index of digestibility, shown in Table 2 as the Least Squares (LS) mean pH for each processed/unprocessed grain. Thus, the LS mean pH values describe the digestibility of a particular cereal at a chosen process condition. Missing data indicate cereals that were unprocessable at particular extrusion conditions.

Table 2 reveals a general trend toward improved in-vitro protein digestibility with extrusion at the 15% moisture condition for each cereal. The LS mean pH values for the extrusion conditions involving 15% feed moisture were consistently lower, revealing higher protein digestibility for these conditions. Although exceptions were noted for high tannin sorghums and wheat, all cereals behaved quite similarly with regard to extrusion effects on protein digestibility. Therefore, interactions involving cereal type with extrusion conditions were of interest so that trends across all seven cereals could be ascertained. To evaluate these overall trends a statistical evaluation of the factors affecting in-vitro protein digestibility across all cereals was performed. The results of interactions involving extrusion condition by cereal are presented in Table 3.

Unprocessed cereals

Results of in-vitro protein digestibility of cereals in the unprocessed state are shown in Fig. 2. Corn, millet, quinoa, low and high tanning sorghum were not significantly ($p > 0.05$) different from each other with regard to initial in-vitro protein digestibility. Rye and wheat were significantly more protein-digestible than any other cereals in the unprocessed state but did not significantly ($p > 0.05$) differ from each other with regard to in-vitro protein digestibility.

Morphological, chemical or functional similarities between cereals may have, in part, been responsible for similar results seen between unprocessed cereals with regard to in-vitro protein digestibility but it was

Table 2. Index of digestibilities for unprocessed and extruded cereal grains

	Corn	Millet	Rye	Quinoa	Low tannin sorghum	High tannin sorghum	Wheat
Unprocessed[d]	6·72^{abc}	6·73^{ab}	6·61^{a}	6·70^{ab}	6·71^{a}	6·71^{b}	6·60^{a}
15% moisture[e] 80°C/100°C, 100 rpm	6·68^{bc}	6·68^{bc}	—	6·71^{ab}	—	—	6·57^{ab}
15% moisture[e] 80°C/100°C, 150 rpm	6·70^{abc}	6·68^{bc}	6·55^{ab}	6·69^{ab}	6·60^{c}	6·72^{ab}	6·53^{bc}
15% moisture[e] 100°C/150°C, 100 rpm	6·65^{c}	6·64^{c}	6·54^{b}	6·65^{b}	6·57^{c}	6·72^{ab}	6·54^{bc}
15% moisture[e] 100°C/150°C, 150 rpm	6·68^{bc}	6·70^{bc}	6·53^{b}	6·69^{ab}	6·62^{bc}	6·72^{ab}	6·50^{c}
25% moisture[e] 80°C/100°C, 100 rpm	6·76^{a}	6·72^{ab}	6·57^{ab}	6·72^{a}	6·65^{abc}	6·77^{a}	6·53^{bc}
25% moisture[e] 80°C/100°C, 150 rpm	6·72^{abc}	6·73^{ab}	6·56^{ab}	6·70^{ab}	6·64^{abc}	6·74^{ab}	6·54^{abc}
25% moisture[e] 100°C/150°C, 100 rpm	6·74^{ab}	6·74^{ab}	6·54^{b}	6·71^{ab}	6·66^{abc}	6·75^{ab}	6·52^{bc}
25% moisture[e] 100°C/150°C, 150 rpm	6·75^{ab}	6·79^{a}	6·57^{ab}	6·66^{ab}	6·68^{ab}	6·77^{a}	6·52^{bc}

Index of digestibility is the Least Square (LS) mean pH for each condition. LS means are based on average pH values of the last four minutes of cereal–enzyme incubation (minutes 7, 8, 9 and 10). Mean values with the same superscripts within the same columns are not significantly different. Least Significant Differences: corn = 0·0726; millet = 0·0736; rye = 0·0605; quinoa = 0·0598; low tannin sorghum = 0·0775; high tannin sorghum = 0·061; wheat = 0·0662; $p < 0·05$.
[d]Average of three replicates.
[e]Average of two replicates.

beyond the scope of this study to confirm this. Data provided on in-vitro protein digestibility of unprocessed cereals used in this study served mainly as a basis of comparison in which to assess digestibility improvements after extrusion processing.

Moisture effects

Feed moisture had a significant effect ($p < 0·01$) by cereal in this study (Table 3). The effect of extrusion feed moisture on in-vitro protein digestibility is shown in Fig. 2. Corn, millet, and low tannin sorghum were all significantly ($p < 0·05$) improved with regard to protein digestibility when extruded at the lower moisture content of 15%. These cereals all had 15% extrusion feed moisture as a significant ($p < 0·05$) main effect influencing in-vitro protein digestibility.

Although low moisture, high temperature processing has been associated with decreased protein nutritional quality (Noguchi *et al.*, 1982; Bjorck *et al.*, 1983) mainly due to lysine losses via the Maillard reaction, these results were not observed for this study. Extrusion temperatures used were probably not high enough when coupled with lower moisture to result in significant hydrolysis needed for the Maillard reaction, which has been shown to require low moisture (<15%) and high temperature (>180°C) (Camire *et al.*, 1990). Lower moisture during extrusion may have also had a 'protective' effect by increasing the viscosity of the system, thus, lowering the reaction rate of degradative processes (Phillips, 1989).

Extrusion temperature has been shown to improve in-vitro protein digestibility of low tannin varieties of sorghum more so than the effects of feed moisture

Table 3. Statistical analysis. In-vitro protein digestibility effect of extrusion conditions across all cereals

Source	Degrees of freedom	*F* value	*P* value
Moisture	1	36·40	0·000 1
Temperature	1	3·21	0·079 2
rpm	1	0·18	0·673 4
Temperature × moisture	1	5·54	0·022 4
rpm × temperature	1	7·45	0·008 7
Moisture × cereal	6	5·54	0·000 2
Temperature × moisture × cereal	6	0·21	0·973 5
rpm × temperature × cereal	6	0·56	0·757 0

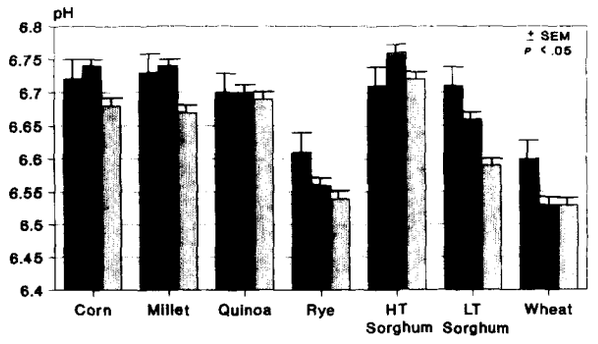

Fig. 2. In-vitro protein digestibility of unprocessed cereals. Effect of moisture on in-vitro protein digestibility of extruded cereals. pH values are Least Squares (LS) means indicating an index of digestibility. LS means were calculated over the last four minutes of cereal–enzyme incubation (minutes 7, 8, 9 and 10). ■ = Unprocessed cereal; ▨ = 25% moisture; □ = 15% moisture.

(15 and 25%) (Fapojuwo *et al.*, 1987) but corn gluten extruded at 14% moisture had higher protein digestibility than that extruded at 20 or 26% feed moisture (Bhattacharya & Hanna, 1988).

In the present study, improved in-vitro protein digestibility observed for corn, millet and low tannin sorghum when extruded at 15% feed moisture was significant ($p < 0.05$) when compared to unprocessed counterparts (Fig. 2).

The effect of moisture also significantly ($p < 0.05$) influenced protein digestibility of high tannin sorghum (Fig. 2) but this finding occurred at 25% feed moisture and was observed as a significant decrease in digestibility (increased pH) when compared to unprocessed high tannin sorghum. The best result for improved in-vitro protein digestibility of high tannin sorghum was when this cereal was extruded at 15% feed moisture (Fig. 2). Even at this condition, in-vitro protein digestibility was not significantly improved from in-vitro protein digestibility of high tannin sorghum in the unprocessed state. The protein digestibility of this cereal did not significantly improve over that of the unprocessed cereal with any form of extrusion processing used in this study. These results support research by MacLean *et al.* (1981) in which it was concluded that other whole-grain cereals are a better source of energy and protein than whole-grain sorghum. Sorghum proteins behave differently from proteins of other cereals after processing, most likely because of formation of disulfide linkages involving kafirin proteins making them less susceptible to enzymatic degradation. Kafirins are also known to be encased in protein bodies which have been observed still intact after cooking and pepsin digestion (Hamaker *et al.*, 1987).

Improvement observed for in-vitro protein digestibility of quinoa, rye and wheat was not influenced by moisture. Although not significant ($p > 0.05$), there was a slight trend favouring improved protein digestibility at the lower feed moisture of 15% for quinoa and rye (Fig. 2).

Although not all cereals had 15% feed moisture as a main effect positively influencing protein digestibility, there was a general trend in this direction. In all cases, except high tannin sorghum and wheat, cereals extruded at 25% feed moisture level did not significantly ($p > 0.05$) differ in protein digestibility from that of the unprocessed cereals (Fig. 2).

Temperature–moisture interactions

There was a significant ($p < 0.05$) interaction between extrusion temperature and feed moisture that influenced in-vitro protein digestibility. As shown in Table 3, this interaction was significant when considered across all cereals studied. Cereals extruded at product temperatures of 100°C/150°C (feed and compression zone temperatures, respectively) and 15% feed moisture, were significantly ($p < 0.05$) more protein-digestible than cereals extruded at alternative product temperature–moisture combinations (data not shown).

In general, heating improves digestibility of proteins by denaturing the protein, exposing new sites for enzyme attack. Heating also aids protein digestibility by inactivating enzyme inhibitors (Lorenz *et al.*, 1980; Rackis *et al.*, 1986). Increasing barrel temperature during extrusion has been shown to increase digestibility of corn gluten–whey blends (Bhattacharya & Hanna, 1988), fish–wheat blends (Bhattacharya *et al.*, 1988), low tannin sorghum (Fapojuwo *et al.*, 1987) and corn grit–quinoa blends (Coulter & Lorenz, 1991). These results correlate well with results of this study, which also demonstrate the beneficial affect of temperature increase on protein digestibility.

Although the influence of moisture appeared to contribute more to improved digestibility (F value for moisture = 36·4) than the temperature increase (F value for temperature = 3·21) (Table 3), the higher temperature–lower moisture processing combination was overall most favourable to digestibility.

Rye and wheat were two cereals in this study that demonstrated improved protein digestibility due to temperature effects, alone. These results (data not shown) were noted at extrusion product temperatures of 100°C/150°C and were significant for rye ($p < 0.05$).

rpm–temperature interactions

Significant ($p < 0.05$) rpm–temperature influences on in-vitro protein digestibility were observed when considered across all cereals studied (Table 3). Across all cereals studied, it was observed that extrusion at 100 rpm and product temperatures of 100°C/150°C resulted in the lowest pH value (data not shown). These results can be attributed to processing at appropriately high extrusion temperatures and long enough residence times, allowing denaturation of proteins to occur without resulting in high-shear environments detrimentally affecting bioavailability.

Previous extrusion studies using wheat demonstrated that, in addition to increasing barrel temperatures above 156°C, screw speeds higher than 100 rpm contributed to lysine losses, therefore decreasing protein digestibility (Bjorck & Asp, 1984). A quinoa–corn grit blend also demonstrated improved in-vitro protein digestibility when extruded at screw speeds of 100 rpm and barrel temperatures of 100–150°C when compared to extrusion at the same barrel temperatures and increased screw speeds of 150 rpm (Coulter & Lorenz, 1991).

CONCLUSIONS

Independent of grain type, effects of extrusion on in-vitro protein digestibility were similar across all cereals studied. Extrusion feed moisture of 15% appeared to strongly influence in-vitro protein digestibility. This effect was somewhat dependent on cereal but a general trend was noted. When 15% feed moisture was coupled with product temperatures of 100°C/150°C, a signifi-

cant ($p < 0.05$) improvement in protein digestibility was observed across all cereals studied. Although effects of rpm were less obvious, when combined with product temperatures of 100°C/150°C, screw speeds of 100 rpm appeared to also improve in-vitro protein digestibility across all cereals studied.

Therefore, optimum extrusion process conditions for cereals used in this study that resulted in improved protein digestibility, were 15% feed moisture, 100°C/150°C product temperatures and screw speeds of 100 rpm. Quinoa was the only single cereal that resulted in significantly ($p < 0.05$) improved protein digestibility dependent on this particular three-way extrusion combination.

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