Protein Quality of Whole Wheat As Affected by Drum-Drying and Single-Screw Extrusion

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Coker 916 whole wheat flour was made into a simulated whole wheat spaghetti by extrusion cooking (single screw, 50 psi, 93 °C) and a flaked product by drum-drying (152 °C). Both single-screw extrusion and drum-drying resulted in substantial reductions, i.e., ≥5% loss, in several essential amino acids. The extruded product was found to contain 16% less lysine, 10% less threonine, 6% less leucine, and 5% less valine than the original whole wheat, while the drum-dried product contained 20% less isoleucine and 16% less methionine. The available lysine content of the whole wheat flour was significantly increased by drum-drying but not by extrusion processing. Both thermal processes significantly increased protein digestibility, while PERs of the drum-dried flakes (1.66) and unprocessed whole wheat (1.59) were significantly greater than that of the extruded product (1.42).

INTRODUCTION

Single-screw extruders have been used in the food industry for over 50 years beginning in 1935 with the introduction of the pasta press, followed some years later by General Mills' use of the technology to make ready-to-eat (RTE) breakfast cereals (Dziezak, 1989). Although in part replaced in the industry today by twin-screw and collet type extruders, single screw extruders continue to produce a variety of food products including RTE cereals, snack foods, modified starches, beverage and gravy mixes, pet foods, texturized vegetable proteins, and confections (Harper, 1978; Dziezak, 1989). Extrusion technology offers food manufacturers the cost advantage(s) of combining several unit operations: mixing, cooking, conveying, forming, puffing, and drying. In addition, the extrusion process itself has been found to have several desirable effects on raw food ingredients which include: destruction and/or inhibition of pathogens and spoilage microorganisms, inactivation of antinutritional factors such as trypsin inhibitor(s), denaturation of enzymes responsible for deterioration of food quality, and gelatinization of starch (Dziezak, 1989).

However, it remains uncertain whether extrusion has minimal, moderate, or severe effects on some of the essential nutrients in foods, proteins in particular. Some studies have indicated that extrusion processing actually increases protein digestibility and/or biological value (Mustakas et al., 1964; Bressani et al., 1978; Hakansson et al., 1987), while other investigators have reported that extrusion causes moderate to severe reductions in essential amino acids, available lysine, and protein quality as determined by rat bioassays (Aguilera and Kosikowski, 1978; Noguchi et al, 1982; Bjorck et al., 1983; Bjorck and Asp, 1984). Some of the reported differences in nutrient retention can be attributed to variations in extruder type and the processing conditions and composition of raw ingredients, e.g. presence or absence of trypsin inhibitors, levels of reducing sugars, while others are more difficult to reconcile.

The present study was undertaken, therefore, to investigate the effects of a mild to moderate form of extrusion cooking on the protein quality of a commonly extruded food product, whole wheat. The amino acid and available lysine content, apparent digestibility, and protein efficiency ratio (PER) of a single variety of whole wheat were determined following single-screw extrusion and compared to that of drum-dried and raw or milled products.

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MATERIALS AND METHODS

Coker 916, a soft, red, winter wheat used in this study, was grown by the Crop and Soil Environmental Sciences Department of Virginia Polytechnic Institute and State University in Giles County, VA, during the 1989 growing season. Harvested wheat kernels were tempered to 14% moisture and milled into flour using a Brabender Mill, Model Quad Jr. II (Brabender, E. Hackensack, NJ). The entire separated bran and endosperm fractions were then recombined into a homogeneous whole wheat flour.

Preparation of Drum-Dried and Extruded Whole Wheat Products. Whole wheat flour (8.1 kg) was combined with tap water to form a slurry (61% moisture) and drum-dried using an American Drum Dryer (Overton Machine Co., Dowagiac, MI) Model P19, operated at 152 °C, a steam pressure of 55 psig, and a drum speed corresponding to 2.5 rpm. The whole wheat material was dried and removed by the doctor blade in approximately 15 s. The resulting flakes of whole wheat were further dried overnight in a 60 °C oven and then stored at −20 °C in double-wrapped polyethylene bags. Drum-dried flakes were ground into a powder prior to analysis using a Regal Coffee and Spice Mill, No. V505 (Regal Ware Inc., Kewaskum, WI).

Whole wheat flour (8.2 kg) was also made into a dough (45% moisture) and extruded using a single screw (1 in. in diameter) Killion Extruder, Model KL-100, operated at 50 psi with its three heating zones held at 66, 93, and 79 °C, respectively. Screw speed was 59 rpm, average feed rate 62 g/min, and mean residence time 97 s. The extruded product resembled whole wheat spaghetti. This material was dried overnight in a oven at a moderate temperature of 60 °C to avoid further protein degradation and then ground into a powder and stored at −20 °C in cotton sacks until used.

Amino Acid Analysis. The drum-dried and extruded products, as well as unprocessed whole wheat kernels, were analyzed to determine their amino acid composition. Samples of each were first hydrolyzed with 6 N HCl for 22 h at 110 °C under a steady stream of nitrogen. The amino acids in the hydrolyzed wheat samples were then separated and quantified using a Carlo Erba 3A29 amino acid analyzer with norleucine as an internal standard. Since cysteine may be partially destroyed during acid hydrolysis (Pellet and Mather, 1986), it was analyzed separately using the preoxidation method of Moore (1963). Tryptophan was determined following sample hydrolysis for 24

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h at 38 °C and a pH of 7.5 using the enzyme pronase. The tryptophan content of the samples was then quantified as before using the Carlo Erba 3A29 amino acid analyzer.

Available Lysine. The available lysine content of the processed and unprocessed whole wheat samples was determined by a procedure adapted from the dye-binding capacity (DBC) method of Hurrell and Carpenter (1976 and 1981). The procedure is as follows. Preweighed amounts of each sample were added to 50-mL polypropylene bottles; one bottle marked A contained 52 mg of sample and one marked B contained 104 mg. Two milliliters of 2-propanol was added to each bottle followed by gentle swirling to thoroughly wet solid sample particles. Propionic anhydride (0.2 mL) was then added to each B bottle, followed by the addition of 2.0 mL of a 5% (w/v) sodium acetate solution. B bottles were capped and placed on a Dubnoff metabolic shaking incubator (Precision Scientific Co., Chicago, IL) and shaken (156 rpm) at room temperature for 15 min. Twenty milliliters of Udy reagent dye solution (Udy Co., Fort Collins, CO) was added to each A bottle followed by the addition of 2.2 mL of 5% sodium acetate. After shaking, 20 mL of Udy reagent dye solution was also added to each B bottle. All bottles were then capped, vortexed, and placed on the shaker for 60 min.

After shaking, the samples from each bottle were filtered using filter disks and ash-free analytical filter pulp (Schleicher and Schuell, Inc.; Keene, NH). Filtered samples were diluted 1:100 by adding 0.05 mL of the clear supernatant to 4.95 mL of distilled deionized water. Diluted samples were vortexed, and their absorbances were read in plastic cuvettes using a Milton Roy Spectronic 601 set at 480 nm against a water blank. The amount of residual dye in millimoles per liter contained in each sample bottle was obtained from a linear regression curve of working standards (12–17 μmol of dye/L).

Available lysine content was calculated as follows:

$$\text{available lysine (g) per 100 g of protein} = \frac{[(A - B) \times 146.3/1000]}{\%\ \text{protein}}$$

where

A = 3.77 − mmol/L of residual dye (bottle A) per gram of sample

B = 3.77 − mmol/L of residual dye (bottle B) per gram of sample

% protein − decimal percent of protein in the sample as determined by AOAC Method 920.87 (AOAC, 1990)

3.77 = concentration of reagent dye solution in mmol/L

146.3 = molecular weight of lysine

Animal Experiments: Determination of Protein Efficiency Ratio and Apparent Digestibility. The protein efficiency ratio (PER) of the drum-dried and extruded products was determined by AOAC Method 960.48 (AOAC, 1990). Four isocaloric rat diets were prepared: (1) an Animal Nutrition Research Council (ANRC) approved reference casein (ICN Biochemicals; Cleveland, OH) control diet, (2) an unprocessed whole wheat kernel diet, (3) a drum-dried product diet, and (4) an extruded product diet. Diets were formulated to contain 10% protein, 13.6% fat ("Mazola" corn oil), 1% vitamin mix (AIN 76, ICN Biochemicals), 4% mineral mix (AIN 76, ICN Biochemicals), and cornstarch (ICN Biochemicals) to make 100%. Forty 21–23-day-old male weanling Sprague–Dawley rats obtained from Dominion Laboratories of Dublin, VA, were randomly assigned to one of four experimental groups (10 rats per group). Mean initial body weights were equal for the four groups. Rats were individually housed in a room maintained at 22 °C, 30–70% RH, with alternating 12-h periods of light and dark. Rats were fed one of four experimental diets ad libitum for 28 days. To calculate PER, rats were weighed biweekly and weight gain and food consumption were recorded. The PER of each diet was calculated using the following equation:

Table I. Amino Acid Composition of Unprocessed, Extruded, and Drum-Dried Coker 916 Whole Wheat (g/100 g Protein)

amino acid	whole wheat	extruded[c]	drum-dried[c]
	Essential Amino Acids		
lysine	3.01	2.54 (16)	3.17
threonine	3.10	2.78 (10)	3.25
valine	4.28	4.05 (5)	4.67
methionine	1.46	1.51	1.23 (16)
isoleucine	3.19	3.17 (1)	2.55 (20)
leucine	7.20	6.75 (6)	6.95 (3)
phenylalanine	4.28	4.29	4.75
tryptophan	1.01	1.08	1.12
	Nonessential Amino Acids		
histidine	2.64	2.62 (1)	2.81
arginine	5.65	4.52 (20)	5.19 (8)
aspartic acid	5.01	4.52 (10)	5.37
serine	4.83	4.60 (5)	5.19
glutamic acid	34.64	30.79 (11)	35.18
proline	10.48	10.40 (1)	10.64
glycine	4.74	4.37 (8)	4.40 (7)
alanine	4.01	3.65 (9)	3.61 (10)
tyrosine[b]	2.73	2.70 (1)	3.17
cystine[a,b]	2.83	2.38 (16)	2.81 (1)
ammonia	4.19	4.52	4.49

[a] Protected cystine method. [b] Tyrosine and cystine have a sparing effect on phenylalanine and methionine, respectively, and therefore can be considered semi-essential. [c] The percentage of amino acid loss due to processing is given in parentheses.

$$\text{PER} = \text{weight gain (g)/protein intake (g)}$$

The PER values obtained using the above equation were corrected as suggested by Chapman et al. (1959):

$$\text{corrected PER} = \frac{2.5}{\text{PER determined for the reference casein diet}} \times \text{PER determined for the test diet}$$

Apparent in vivo digestibility of the four rat diets was determined by the method of Gumbmann et al. (1983). Using this method apparent digestibility was based on the amount of diet consumed and Kjeldahl nitrogen analysis of feces collected on an individual basis for a period of 8 days (14th–21st day of the PER study). Apparent in vivo digestibility was calculated as follows:

$$\%\ \text{apparent digestibility} = \frac{N\ \text{intake (g)} - \text{fecal}\ N\ \text{(g)}}{N\ \text{intake (g)}} \times 100$$

where

N intake = diet consumed for 8 days × (% N in diet/100)

and

fecal N = (% N in feces/100) × fecal weight (g)

Statistical Analysis. Statistical analyses (means, standard deviations, and Fisher's LSD) were conducted using the Number Cruncher Statistical System, version 5.0 (Hintze, 1987).

RESULTS AND DISCUSSION

The amino acid composition of Coker 916 whole wheat, extruded, and drum-dried products is given in Table I. Both thermal processes resulted in substantial reductions, i.e., ≥5% loss, in several essential and nonessential amino acids. The extruded product was found to contain 16% less lysine, 10% less threonine, 6% less leucine, and 5% less valine than the original whole wheat, while the drum-dried product contained 20% less isoleucine and 16% less methionine. Some destruction of all 10 nonessential amino acids also occurred as a result of extrusion processing with losses exceeding 10% in the case of arginine, glutamic acid, and cysteine. Drum-drying caused less destruction of nonessential amino acids than single-screw extrusion,

resulting in >5% loss of only three amino acids: arginine, glycine, and alanine.

Similar results were reported by Bjorck et al. (1983), who found that extruded cereal based biscuits lost up to 38% of their total lysine, 14% of cysteine, and 21% of arginine. Cubadda (1988) also reported substantial reductions in total lysine (24%), methionine (20%), arginine (18%), and cysteine (11%) as a result of extrusion cooking of millet-gram flour. Other investigators have found that both drum-drying and extrusion cooking can have detrimental effects on the amino acid content of processed foods. Aguilera and Kosikowski (1978) reported that a corn–soy–whey mixture lost 5% of its lysine, 15% of its methionine, and 12% of its cysteine as a result of drum-drying and up to 15% lysine, 6% methionine, 14% cysteine, and 26% arginine following extrusion processing. Maga and Sizer (1979) observed that the free amino acids in raw potato were rather rapidly destroyed during drum-drying (losses ranging from 19 to 86%) and extrusion cooking (30–98% loss).

The extrusion process caused greater destruction of amino acids present in the original whole wheat than did drum-drying despite the fact that extrusion was carried out at a lower temperature (93 °C) than was drum-drying (152 °C). However, the extruded product was heated longer (mean residence time of 97 s) than its drum-dried counterpart (dried in less than one rotation of the drum or within 15 s). The destruction of nutrients like that of microorganisms is governed by a time–temperature relationship. High temperature–short time thermal processing of foods often results in greater nutrient retention than processes employing lower temperatures but longer heating times (Karmas, 1975).

Destruction of lysine and other amino acids may have been strongly influenced by the moisture content/water activity level of the whole wheat products during various stages of thermal processing. Lysine and other essential amino acids are susceptible to Maillard and Strecker degradations, reactions which are favored at high temperatures and immediate moisture levels (Hodge and Osman, 1976).

During drum-drying the moisture content of a whole wheat flour slurry was rapidly reduced from 61 to 15% moisture which means that the slurry was held for only a few seconds at the optimum water activity level required for Maillard browning. The whole wheat remained at a higher moisture content immediately after extrusion (40%). The moisture content of the extruded product was then slowly reduced to 5% by oven-drying at 60 °C. Thus it is likely that the extruded product was subjected to intermediate moisture/water activity levels that favor Maillard browning for a much longer time period than the drum-dried product. The loss of cysteine in the extruded product may also be attributed to the high moisture content of the whole wheat following extrusion. Free water is required for the reduction reaction that splits the disulfide bridge of cysteine yielding two cysteine residues which may have then undergone further thermal degradation (Angelmier and Montgomery, 1976). The free water necessary for this reaction to occur was present in greater amounts in the extruded than in the drum-dried product.

It is unclear why drum-drying caused greater destruction of methionine and isoleucine than did single-screw extrusion. Methionine and isoleucine are known to be converted to methional and 3-methylbutanol by Strecker type degradation reactions that normally accompany Maillard browning (Eichner and Ciner-Doruk, 1981; Maga and Sizer, 1979). The investigations of Eichner and Ciner-Doruk (1981) indicated that the rate of Strecker degradation of α-amino acids is accelerated in slightly moist rather than completely dry food products. The drum-dried slurry had a higher original moisture content (61%) than the extruded dough (45%) which could have been a critical determinant in regard to how much methionine and isoleucine were retained in the two products.

Table II. Available Lysine Content[a] of Milled, Extruded, and Drum-Dried Coker 916 Whole Wheat (g/100 g Protein)

sample	available lysine	% total lysine
milled MW	2.30 ± 0.055 a	76.4
extruded	2.19 ± 0.101 a	72.8
drum-dried	2.64 ± 0.134 b	87.7

[a] Mean values followed by the same letter are not significantly different ($\alpha = 0.05$), Fisher's LSD test. Means and standard deviations of six separate trial runs on each sample.

Table III. PER, Apparent in Vivo Digestibility, and Feed Intake of Casein Control, Unprocessed, Drum-Dried, and Extruded Coker 916 Whole Wheat Diets[a]

rat diet	adjusted PER	apparent *N* digestibility, %	feed intake, g
casein control	2.50 ± 0.28 a	92.4 ± 1.71 a	444.4 ± 32.0 a
unprocessed	1.59 ± 0.08 bc	82.5 ± 2.04 d	440.7 ± 42.2 a
drum-dried	1.66 ± 0.22 b	90.0 ± 1.63 b	432.9 ± 40.6 a
extruded	1.42 ± 0.12 c	87.2 ± 1.54 c	311.8 ± 28.5 b

[a] Mean values in the same column followed by the same letter(s) are not significantly different ($\alpha = 0.05$), Fisher's LSD Test. Based on 10 rats per dietary treatment.

The results of the available lysine analysis are presented in Table II. The available lysine content of the three whole products was determined using the dye-binding capacity (DBC) method of Hurrell and Carpenter (1976). It has been reported (Hurrell and Carpenter, 1981; Finot and Hurrell, 1985) that in foods containing early Maillard browning reaction products, the dye binding procedure overestimates the reactive lysine, because the dye reacts with the basic deoxyketosyl lysine derivative, which is biologically unavailable. This would not be a problem if the dye still reacted with the deoxyketosyl lysine after propionylation. However, this has not been confirmed (Hurrell and Carpenter, 1981). Finot and Hurrell (1985) reported that about 30% of the Amadori compound of lysine was propionylated like reactive lysine.

Taking into consideration the possibility of overestimation of available lysine by the DBC method, the amount of available lysine was less than the total lysine for all three samples. The observed percent of total lysine for whole wheat flour (76.4%) agrees with the result reported by McAuley et al. (1987) (77.4%) determined by a modified TNBS method. The drum-dried product had a significantly greater amount of available lysine than the extruded product and the whole wheat flour. The higher available lysine value of the drum-dried product is an indication that drum-drying resulted in some thermal denaturation and/or dissociation of wheat flour proteins making them more susceptible to Udy dye binding, while the decrease in the available lysine content of the extruded product suggests that in addition to thermal denaturation/dissociation extrusion processing may also have caused some degree of protein cross linking (Anglemier and Montgomery, 1976) with loss of dye binding capacity.

The PER, apparent in vivo digestibility, and feed intake of the casein control and three whole wheat diets are reported in Table III. The PER values were adjusted or standardized as suggested by Chapman et al. (1959). The PER of the ANRC casein control was significantly greater than any of the Coker 916 whole wheat diets. The PERs

of the casein control and the drum-dried product were significantly greater than that of the extruded product. The observed increase in the protein quality of Coker 916 whole wheat following drum-drying may have been due to disruption of tertiary and quaternary protein structure, thus increasing the susceptibility of these proteins to enzymatic attack. This, in turn, would affect the rate of release and absorption of amino acids from the small intestine of the rat. The PER of the extruded product was significantly less than the drum-dried product but not significantly different from the PER of the unprocessed whole wheat kernels. This may be due to the loss of essential amino acids, especially the limiting amino acid lysine, that occurred during the extrusion process.

Apparent in vivo digestibility indicates the relative percentage of nitrogen absorbed by the rat from each diet. Both drum-drying and single-screw extrusion resulted in significant increases in apparent digestibility of Coker 916 whole wheat. Drum-drying was significantly more effective than extrusion, however, in improving nitrogen bioavailability. The unprocessed whole wheat had a significantly lower digestibility than the other three diets. Although both thermal processes had positive impacts upon digestibility, intestinal release and absorption of nitrogen from the extruded diet may have been somewhat impaired by the formation of undigestible cross-linked and polymerized products of Maillard browning.

Rats consuming the extruded diet had significantly lower feed intakes than rats in the other diet groups (see Table III). Mitchell (1927) was perhaps the first to recognize that the growth rate of rats was influenced by their feed intake. She questioned the still common practice of drawing experimental conclusions based on the composition of the diet alone without considering the amount of food consumed by laboratory animals. More recently Knipfel (1981) reported that weight gains in rats fed autoclaved casein, soy, egg, or fish protein diets decreased markedly due to depressed caloric intake. It was also reported by Harper and Peters (1989) that food intake of animals depends on the protein content and the amino acid balance of the diet.

Characteristic signs of an amino acid imbalance in the diet include depression of food intake and growth (Harper, 1964; Harper and Peters, 1989). Leung and Rogers (1987) observed that when rats are offered a choice between diets they will select a protein-free diet or a relatively balanced amino acid diet over a diet with an imbalance of amino acids. They proposed that specific mechanisms exist that provide feedback control for depression of food intake when animals ingest diets that would drastically alter their amino acid pools. It seems likely, therefore, that the rats fed the extruded diet decreased their food intake to avoid an amino acid imbalance. However, the palatability of the diets can also not be ignored. Rats fed the extruded product diet may have consumed less feed simply because they disliked the taste.

The results presented here are in general agreement with a number of other studies which indicate that extrusion processing can, in some cases, have detrimental effects on essential amino acids in foods and, hence, protein quality. It is surprising how little is known, however, about the fate of essential amino acids during the extrusion process given the possible severity of the effects and their impact on protein bioavailability for humans. Further research should focus, therefore, on (1) delineation of the specific reactions responsible for degradation of essential amino acids during both single- and twin-screw extrusion and (2) identifying processing conditions, i.e. screw speed, heating zone temperatures, etc., which result in maximum retention of amino acids and other essential nutrients.

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Registry No. Lysine, 56-87-1; threonine, 72-19-5; leucine, 61-90-5; valine, 72-18-4; isoleucine, 73-32-5; methionine, 63-68-3.