Effect of the Hard-to-Cook Defect and Processing on Protein and Starch Digestibility of Cowpeas

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ABSTRACT

Cowpeas were stored for six weeks at 37°C and 85% rh for a test group and at 7°C and 60% rh for a control group. After storage, they were treated as follows: cooked for 45 min; cooked for 90 min; ground into a flour; made into a flour slurry (20% solid) and cooked as a gruel for 45 min; or ground into flour and extruded at 20% moisture with a barrel temperature of 150°C. The hard-to-cook defect was observed in the test group. The effect of storage conditions and processing treatments on the nutritional quality of cowpea protein and starch was determined by in vitro and in vivo (fecal and ileal digestibility) methods. The in vitro and ileal methods indicated that the protein digestibility of the control group was significantly higher than that of the test (hard-to-cook) group (78.5 vs. 75.3% and 76.9 vs. 66.6%, respectively); the fecal assay detected no difference. In vitro and ileal protein digestibility was highest in cowpeas cooked for 45 min (80.3 and 81.8%, respectively) and lowest in cowpeas ground into flour (72.6 and 65.4%, respectively). Grinding before processing exacerbated the differences between the control and the test groups. Starch digestibility was enhanced by grinding before cooking but showed little response to storage conditions.

Cowpea (*Vigna unguiculata*) is one of the most important grain legumes in diets in developing countries, especially in West Africa. In the Nsukka area of eastern Nigeria cowpea purchases varied from 170 to 775 g per week, and consumption ranged from once per week (12% of the households) to three times per week (35%) or more (23%). Cowpeas are also used extensively as a weaning food; almost one third of mothers introduce them as a first food for infants six months of age or younger (King et al 1985). In 1975, it was estimated that 60% of dietary protein in the former Western state of Nigeria was derived from cowpeas. They are an important source of calories and vitamins as well as protein. The seed is 25% protein and 65% carbohydrate and contains thiamin, riboflavin, and niacin (0.74, 0.42, and 2.81 mg/100 g, respectively) (Bressani 1985). Cowpeas are extremely versatile: they can be incorporated into soups and stews or can be decorticated, ground into paste, and steamed to make *moin-moin* or fried to make *akara* (Dovlo et al 1976).

One of the major factors limiting expanded consumption of legumes is storage-induced textural defects, which prolong cooking time and demand correspondingly higher fuel inputs (Sefa-Dedeh et al 1979). A recent survey of consumers in Nigeria revealed that cooking difficulty was the leading constraint on cowpea consumption (Uwaegbute and Nnanyelugo 1987). The hard-to-cook (HTC) defect is the failure of leguminous seeds to become soft enough to eat after cooking for a reasonable time. It is caused by storage of beans and peas at elevated temperature and relative humidity and is characterized by a failure of cells to separate following cooking (Jones and Boulter 1983). The proposed mechanisms have been extensively reviewed by several investigators (Aguilera and Stanley 1985; Hincks and Stanley 1986, 1987). These mechanisms may be briefly characterized as 1) insolubilization of middle-lamella pectins by divalent ions released by phytase and 2) oxidative cross-linking of phenolic compounds in the cell wall.

In addition to reducing palatability and wasting fuel, HTC may compromise the nutritional quality of legume seeds. Molina et al (1975) found a negative relationship between storage and the protein digestibility, protein efficiency ratio, and protein solubility of black bean stored at 25°C for three and six months. Antunes and Sgarbieri (1979) reported that storage of dry beans at high temperature (37°C) and high relative humidity (76%) not only had a severe effect on the rate of hardening but also lowered the protein quality and the availability of essential amino acids. Sievwright and Shipe (1986) demonstrated marked increases in hardness and decreases in digestibility in vitro, accompanied by changes in tannins and phytates, in black beans stored at 30 or 40°C and 80% rh. Uma Reddy and Pushpamma (1986) reported a 10–30% loss of lysine, methionine, and tryptophan in green gram, pigeon pea, and chickpea, as well as decreases in net protein ratio and digestibility.

The object of this study was to assess effects of the HTC defect and various cooking methods on the digestibility of protein and starch in cowpeas.

MATERIALS AND METHODS

Cowpea Preparation

Cowpea seeds (*V. unguiculata* cultivar California Blackeye No. 5, a gift from Kerman Warehouse, Kerman, CA) serving as a control and those awaiting study were stored at 7°C and 60% rh until used. Seeds subjected to accelerated storage conditions were dipped in a solution of 0.05% potassium sorbate/sorbic acid (pH 5.5) and 70% ethanol to prevent mold growth and were air-dried under cheesecloth at room temperature (25–27°C). Treated seeds were placed in sterilized plastic containers, adjusted to 25% moisture by weight (Pappas and Rao 1987), and stored at 37°C for six weeks with saturated KCl to maintain 85% rh.

Processing Treatments

After storage, test cowpeas were air-dried overnight at room temperature. Control and test samples were divided into five 4.54-kg groups for further processing.

Treatment A. Seeds were soaked for 16 hr in a volume of distilled water equivalent to four times their weight, drained, and then added to the same volume of boiling water in a steam-jacketed kettle. Boiling was continued for 45 min. The cooked seeds were drained immediately, allowed to cool in an ice water bath, and then drained again for 30 min. A sample of 150 g of cooked seeds was taken for texture measurement. The remainder was passed through a sausage grinder twice, freeze-dried, gently pulverized by a mortar and pestle, and stored at 7°C until further study.

Treatment B. Seeds were boiled for 90 min, in exactly the same procedure as in treatment A.

Treatment C. Seeds were ground into flours in a Retsch centrifugal grinding mill (model ZM1, Retsch GmbH, Haan, West Germany) equipped with a 0.2-mm screen. The flours were stored at 7°C until used.

Treatment D. Cowpea flour (4.54 kg) was slurried in four times its weight in water. The slurry was brought to boiling in a steam kettle and heated for 45 min after boiling began, with constant stirring; water was added to maintain the volume. The cooked paste was freeze-dried and ground in the Retsch mill.

Treatment E. Cowpea flour (4.54 kg) was hydrated to 20% moisture (w/w) by adding water to the flour while mixing it in

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a Hobart mixer (model A-200, Hobart Corporation, Troy, OH). After storage overnight at 7°C, the hydrated flour was extruded through a Yellow Jacket pilot scale extruder (Wayne Machine and Die Co., Totowa, NJ) as described by Phillips et al (1984). Extrusion was performed at 150°C with the screw speed held constant at 180 rpm. The temperature of the product exiting from the extruder was 110°C. Extrudates were collected, air-dried, and then ground in the Retsch mill.

Physical and Chemical Analyses

The texture (degree of hardness) of 50 g of seeds boiled for either 45 or 90 min was evaluated by an Instron Universal Testing Machine equipped with a Kramer test cell and a 500-kg load cell and having a crosshead speed and a chart speed of 50 mm/min each. The peak force required to shear and compress the cooked seeds per unit of mass of the sample (N/g) was calculated to represent the hardness.

The moisture content of all processed samples was determined by the AOAC vacuum oven method (method 14.002, AOAC 1980). The protein content was determined by macro-Kjeldahl (method 7.025, AOAC 1980), with the nitrogen content multiplied by the conversion factor 6.25. The starch content of processed cowpea, diet, ileum, and feces samples was determined by modified methods of Budke (1984) and Bjorck et al (1986). Starch was gelatinized either by autoclaving (cowpea and fecal samples) or by exposure to 4N KOH (ileal and diet samples). Gelatinized starch was converted to glucose by treatment with bacterial α-amylase and fungal amyloglucosidase (Sigma Chemical Co., St. Louis, MO). Glucose was quantified with a YSI analyzer (model 27, Yellow Springs Instrument Co., Yellow Springs, OH) equipped with a glucose oxidase membrane. The mean moisture and the protein and starch contents of the 10 processed, freeze-dried cowpea samples are shown in Table I. Chromium, used as a nondigestible marker for determining ileal digestibility, was quantified by inductively coupled plasma spectrography (model P-40, Perkin-Elmer Corp., Norwalk, CT) following the digestion of samples with nitric acid/perchloric acid as described by the manufacturer.

TABLE I
Mean Moisture, Protein, and Starch Contents of Cowpeas Processed After Storage

Cowpea Sample and Storage Group[a]	Moisture (%)	Protein (%)[b]	Starch (%)[b]
Seeds boiled 45 min			
C	3.01 ± 0.01	21.65 ± 0.06	51.07 ± 0.43
T	4.22 ± 0.03	26.25 ± 0.06	54.42 ± 0.62
Seeds boiled 90 min			
C	4.82 ± 0.04	22.75 ± 0.03	50.59 ± 0.48
T	7.46 ± 0.01	26.19 ± 0.06	53.92 ± 1.82
Paste			
C	3.97 ± 0.02	25.61 ± 0.17	44.59 ± 0.71
T	4.10 ± 0.06	25.88 ± 0.13	47.40 ± 0.50
Extrudate			
C	10.41 ± 0.06	24.31 ± 0.22	47.47 ± 0.93
T	8.30 ± 0.01	26.32 ± 0.07	47.87 ± 1.52
Flour			
C	10.00 ± 0.02	25.64 ± 0.23	47.72 ± 1.30
T	11.33 ± 0.06	25.94 ± 0.16	49.60 ± 0.91

[a] C = control group, stored at 7°C and 60% rh; T = test group, stored at 37°C and 85% rh. The cowpeas were stored for six weeks.
[b] Dry weight basis.

In Vitro Digestibility

In vitro protein digestibility was determined by the four-enzyme method of Satterlee et al (1982). The percent protein digestibility of each sample was determined by the following equation: Percent protein digestibility = 234.84 − 22.56(X), where X is the pH at 20 min. Starch digestibility was determined on the basis of maltose released after sequential amylolysis with pepsin and pancreatin, in a modification of the method described by Kon et al (1971): Percent starch digestibility = $(M \times 50 \times 100)/(W \times S)$, where M is the maltose concentration of the sample (mg/ml), W is the sample weight (mg), and S is the percent starch in the original sample.

In Vivo Study

Male weanling (21-day-old) Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) were maintained on a stock diet until 24 days of age and then weighed; 20 animals at the extreme weights were discarded. The others were distributed into 24 groups of five so that the groups varied in mean body weight (~50 g) by less than 1 g and the standard deviation of the mean was essentially the same in all groups. Twenty groups were randomly assigned to the 10 cowpea diets, two groups to a casein diet, and one group to a protein-free diet; one group was sacrificed at the start of the experiment.

The composition of the diets is shown in Table II. Cowpeas served as the protein and starch source in the first 10 diets. The control casein diet contained casein and L-methionine (0.015 g/g of casein) and was supplemented with cornstarch instead of cowpeas. These 11 diets were formulated to be isonitrogenous (2.4% nitrogen). The protein-free diet was used to estimate the

TABLE II
Composition of Diets (%)

Diet[a]	Cowpea	Sucrose[b]	Oil[b]	Salt Mix[c]	Vitamin Mix[d]	Casein[d]	Cornstarch[b]	Celufil[d]
Cowpea								
Seeds boiled 45 min								
C	58.8	31.7	5.0	3.5	1.0	...	...	...
T	59.7	30.8	5.0	3.5	1.0	...	...	...
Seeds boiled 90 min								
C	59.5	31.0	5.0	3.5	1.0	...	...	...
T	61.9	28.6	5.0	3.5	1.0	...	...	...
Paste								
C	61.0	29.5	5.0	3.5	1.0	...	...	...
T	60.4	30.1	5.0	3.5	1.0	...	...	...
Extrudate								
C	69.4	21.1	5.0	3.5	1.0	...	...	...
T	62.1	28.4	5.0	3.5	1.0	...	...	...
Flour								
C	65.0	25.5	5.0	3.5	1.0	...	...	...
T	65.2	25.3	5.0	3.5	1.0	...	...	...
Casein	...	28.2	5.0	3.5	1.0	17.2	42.0	3.1
Protein-free	...	28.2	5.0	3.5	1.0	...	59.2	3.1

[a] C = control group of cowpeas, stored at 7°C and 60% rh; T = test group, stored at 37°C and 85% rh. The cowpeas were stored for six weeks.
[b] Sucrose, oil (100% corn oil), and cornstarch were purchased from a local grocery store.
[c] Williams-Briggs modified mineral mix (catalogue no. 170911, Teklad Test Diets).
[d] AIN Vitamin Mixture 76, Casein-Vitafree, and Celufil Non Nutritive Bulk (catalogue nos. 10663, 12866, and 13292, respectively, United States Biochemical Corporation, Cleveland, OH).

amount of endogenous nitrogen. All the diets were formulated to have the same percentages of vitamin mix, mineral mix, and oil. Each diet was adjusted to 100% with sucrose.

The animals were housed individually in wire cages in a controlled environment (22 ± 1°C and 50% rh) with alternate 12-hr cycles of darkness and light; water was provided ad libitum. Two groups of five rats were assigned to each diet, except the protein-free diet. For the first 14 days, one group was fed 7 g of the diet per day, and the other was fed 10 g per day. These amounts were increased to 8 and 11 g per day, respectively, for the remaining seven days. The group assigned to receive the protein-free diet was fed the casein diet at 10 g per day for the first 11 days and then the protein-free diet at 11 g per day for the final 10 days of the study. Spilled food was collected and weighed.

Fecal Digestibility

For determining fecal digestibility, feces of rats fed 11 g per day were collected, carefully separated from any spilled food, and stored at −20°C from day 16 through day 22. At the end of the study, feces were freeze-dried and then ground in a coffee mill for 1 min. The nitrogen and starch contents of the feces were determined as described earlier. True protein digestibility was calculated from the following equation:

$$\text{Percent true protein digestibility} = [I - (F - F_0)] \times 100/I$$

where I is the nitrogen intake of test protein, F is fecal nitrogen, and F_0 is the fecal nitrogen from rats receiving the protein-free diet.

Ileal Digestibility

On day 23, the rats that had been receiving 10 or 11 g per day were fed 5 g of the assigned diet mixed with 0.35% chromic oxide (as a nondigestible marker). The animals were killed by CO_2 asphyxiation 3 hr after the food was given. The gastrointestinal tract of each animal was removed from the carcass and the distal half of the ileum was dissected from it. The ileal contents were carefully flushed out with distilled water from a plastic syringe and stored in a capped vial at −20°C. The collected ileum samples were freeze-dried, mixed with Celufil protein-free cellulose diluent (United States Biochemical Corporation, Cleveland, OH) and ground for 1 min in a coffee mill. Samples were analyzed for nitrogen, chromic oxide, and starch contents. The protein and starch digestibilities were calculated from the following equations:

$$\text{Percent true protein digestibility} = [I \times R - (N - N_0)] \times 100/(I \times R)$$

$$\text{Percent starch digestibility} = (I \times R - S) \times 100/(I \times R)$$

where R is the percentage of feed intake represented by the ileum sample, i.e., [weight of ileal contents (g) × percent Cr in ileal sample]/[feed consumed (g) × percent Cr in feed]; I is the intake of starch or nitrogen; N is the nitrogen content of the ileal sample; N_0 is the nitrogen content of the ileal sample from rats receiving the protein-free diet; and S is the starch content of the ileal sample.

Statistical Analysis

Results of texture studies and in vitro and in vivo studies were analyzed by the Statistical Analysis System program (SAS 1985) using one-way and two-way analysis of variance (ANOVA) to determine significant main effects (storage conditions and processing treatments) and interactions at an α level of 0.05. When effects of storage conditions and processing treatments were shown, the results were evaluated further using the Duncan's multiple range test. The various methods of assessing the digestibility of cowpeas were compared using the correlation procedure (Proc Corr) of SAS.

RESULTS AND DISCUSSION

Effect of Storage Condition and Cooking Time on Cowpea Texture

The maximum force per unit of mass required to shear-compress cooked cowpea seeds in the Kramer cell is shown in Table III. The effects of storage conditions (7°C and 60% rh for the control group and 37°C and 85% rh for the test group) and cooking time (45 and 90 min) on cooked cowpea texture, as well as their interactions, were significant. The required shear-compression force was significantly higher for the test group than for the control group at both cooking times. Increased cooking time (90 min) reduced the required force in both groups (by 31% in the test group and 13% in the control group). Nevertheless, the force required to shear cowpeas from the test group cooked for 90 min was still five times greater than that for cowpeas in the control group.

Aguilera and Ballivian (1987) observed that when black beans were stored for nine months at various moisture contents and temperatures, hardness increased with moisture content and temperature. Hung and Phillips (*unpublished*) found that both the force and the energy per unit of mass required to shear-compress cowpeas cooked for 45 and 90 min increased with storage temperature and relative humidity. The 7° increase in storage temperature from 37 to 44°C induced a significant increase in hardness, compared to the similar change from 30 to 37°C. When seeds were stored for six weeks at 75% rh and 30, 37, and 44°C and then boiled for 45 min, the forces required to shear-compress them were approximately 18, 22, and 44 N/g, respectively, compared to 9 N/g for the control. These findings are consistent with those in the present study. Hung and Phillips (*unpublished*) also noted that the hardening rate of cowpea seeds that were stored at high temperature and humidity and became HTC can be characterized by a pseudo-zero-order rate law, and hardening at different temperatures was accelerated, as would be expected by Arrhenius theory.

Protein Digestibility

Storage conditions, processing treatments, and their interactions all had significant effects on in vitro protein digestibility

TABLE III
Effect of Storage and Cooking Time on the Shear-Compression Force of Cowpeas

Storage Group	Cooking Time (min)	Force (N/g)[a]
Test	45	42.78
	90	29.39
Control	45	6.88
	90	5.96

[a] All means are significantly different ($P \leq 0.05$).

TABLE IV
Effect of Storage and Processing on In Vitro and In Vivo Protein and Starch Digestibility of Cowpeas

	Protein Digestibility (%)[a]			Starch Digestibility (%)	
	In Vitro	Fecal	Ileal	In Vitro	Ileal
Storage group					
Control	78.50 a	84.79 a	76.86 a	69.90 b	89.70 a
Test	75.25 b	82.34 a	66.59 b	76.98 a	90.02 a
Processing treatment					
Seeds boiled 45 min	80.30 a	87.55 a	81.79 a	60.87 d	89.82 a
Seeds boiled 90 min	79.10 b	86.73 a	70.37 bc	67.52 c	90.16 a
Paste	74.85 d	85.79 a	75.61 ab	84.77 b	90.62 a
Extrudate	77.49 c	85.25 a	65.48 c	92.19 a	90.58 a
Flour	72.63 e	73.51 b	65.38 c	61.08 d	88.14 a

[a] Storage group means in a column followed by the same letter are not significantly different, and processing treatment means in a column followed by the same letter are not significantly different (both at $P \leq 0.05$).

at an α level of 0.05, as shown in Table IV. The control group had significantly higher digestibility than the test group. When processing treatments were compared, cowpeas boiled for 45 min had the highest digestibility, followed by those boiled for 90 min, extruded cowpeas, cooked paste, and raw flour. The mean values for in vitro protein digestibility of all samples are shown in Table V. In each treatment, digestibility was significantly higher in the control group than in the test group. The difference was greater when cowpeas were ground into flour, cooked as a gruel, and extruded than when whole cowpeas were boiled.

Onayemi et al (1986) reported that in vitro protein digestibility was 61% for freshly harvested cowpeas cooked in a pressure cooker and 48% for fresh cowpeas cooked with 0.3% sodium carbonate. After six months of storage in jute bags, the in vitro digestibility of cowpeas cooked by these methods was 59 and 45%, respectively. The digestibility of all cowpea materials in the present study was higher than that reported by Onayemi et al (1986). This may be due to the different method applied to determine in vitro protein digestibility. Phillips and Baker (1987) reported that the in vitro protein digestibility of processed cowpeas was highest for extruded flour (83–85%), intermediate for *akara* (82.8%) and steamed, drum-dried paste (81.2%), and lowest for raw meal (77.8%). In the present study, the digestibility of extruded cowpeas in the control group (79.9%) and that of raw flour in the control group (74.1%) were both lower than the values reported by Phillips and Baker (1987). The difference, about 4%, may be due to the different cowpea cultivar (Mississippi Silver Hull) used in the previous study. The in vitro protein digestibility of germinated, decorticated, cooked California Blackeye cowpea reported by Nnanna and Phillips (1990) was 81.6%, comparable to that of the control group boiled for 45 min (81.2%) in the present study. Sievwright and Shipe (1986) reported that even storage at 5°C and 50% rh reduced the (pepsin) in vitro protein digestibility of black bean protein from 76 to 70% when the beans were soaked in water and from 82 to 76% when they were soaked in a solution of sodium chloride and sodium carbonate. Storage for six months at 40°C and 80% rh further reduced the digestibility of water- and salt-soaked beans to 61 and 75%.

There was no significant difference between the control and test groups in fecal protein digestibility (Table IV). Cowpea flour was significantly less digestible than cowpeas processed by the other treatments. No significant difference was observed between boiling for 45 min, boiling for 90 min, cooking as paste, and extrusion (Table IV). Among all samples, only cowpea flour was significantly different. Fecal digestibility values were generally higher than in vitro values (Table V). Hopkins (1981) reported that the true protein digestibility of Alaskan field peas by adult humans was 88% and that of isolated soybean protein was 93–97%.

Storage conditions had significant effects on ileal protein digestibility, as shown in Table IV. When processing treatments were compared, cowpeas boiled for 45 min had the highest protein digestibility, followed by cooked paste and cowpeas boiled for 90 min; extruded cowpeas and raw flour had the lowest values (Table IV). The numerical differences were greater for ileal than for in vitro digestibility in most cases. However, significant differences between the control and test groups were obtained only for paste, extruded cowpeas, and flour. In each case, the control samples were more digestible than the test samples (Table V).

The difference observed between ileal digestibility and fecal digestibility should be attributed to the effects of microbial activity and amino acid absorption in the large intestine. According to Slump and van Beek (1975), the amount of amino acid related to a given amount of chromic oxide in different parts of the rat intestine decreased from cecum contents to feces. The decrease is comparable for all individual amino acids. They also found that the decrease of total amino acids was greater than that of total nitrogen, indicating that deamination and absorption of amino acids may occur in the large intestine. Neglecting these facts may result in too-low values for amino acid digestibility when the ileum analysis method is used. The endogenous protein and amino acid levels in the ileum and colon are not well characterized. Amino acid balance is the same for the animal whether the excreted amino acids are coming from its body proteins or from partly hydrolyzed food proteins. Conversely, Sauer et al (1989) cited evidence that amino acids escaping digestion in the ileum make little or no contribution to amino acid nutrition in the pig. Nitrogen from protein or amino acid infused into the terminal ileum was digested and absorbed but excreted quickly and almost completely in the urine. These authors claimed that the ileal method should be considered an improvement over the fecal method.

The in vitro protein digestibility was positively correlated with true (fecal) digestibility, but the correlation between in vitro and ileal data failed to be significant at an α level of 0.05. There was no significant correlation between fecal digestibility and ileal digestibility of protein. This lack of correlation was probably due to the action of colon bacteria on protein transported from the ileum to the colon.

TABLE V
Mean In Vitro and In Vivo Protein and Starch Digestibility of Cowpeas Processed After Storage

Sample and Storage Group[a]	Protein Digestibility (%)[b]			Starch Digestibility (%)	
	In Vitro	Fecal	Ileal	In Vitro	Ileal
Seeds boiled 45 min					
C	81.21 a	87.95 b	83.39 abc	62.57 ef	88.79 ab
T	79.40 c	87.15 b	80.18 bc	59.17 f	90.86 ab
Seeds boiled 90 min					
C	80.08 b	86.38 b	64.93 def	70.91 d	89.02 ab
T	78.12 d	87.08 b	75.82 cd	64.13 e	91.31 ab
Paste					
C	77.15 e	85.02 b	88.03 ab	80.14 c	89.57 ab
T	72.56 h	86.56 b	63.19 ef	89.40 b	91.67 ab
Extrudate					
C	79.93 b	85.74 b	74.01 cde	89.93 b	96.92 a
T	75.04 f	82.75 b	56.95 f	94.46 a	84.23 b
Flour					
C	74.14 g	78.87 bc	73.95 cde	44.43 g	84.20 b
T	71.13 i	68.14 c	56.81 f	77.73 c	92.08 ab
Casein diet	...	99.03 a	93.34 a	...	94.43 ab

[a] C = control group, stored at 7°C and 60% rh; T = test group, stored at 37°C and 85% rh. The cowpeas were stored for six weeks.
[b] Means in a column followed by the same letter are not significantly different ($P \leq 0.05$).

Starch Digestibility

The test cowpea group had significantly higher in vitro starch digestibility than the control group, as shown in Table IV. When processing treatments were compared, extruded cowpeas had the highest digestibility, followed by cooked paste, cowpeas boiled for 90 min, raw flour, and cowpeas boiled for 45 min. The mean values for in vitro starch digestibility of all samples are shown in Table V. The test extrudates had the highest and the control flour had the lowest starch digestibility. The digestibility of the test group was higher than that of the control group, except for cowpeas boiled for 90 and 45 min. Control cowpeas boiled for 90 min had significantly higher digestibility than the test cowpeas. The difference between the test and the control raw flour was greater than the differences between the test and control groups for the other treatments. It is interesting to note that cowpea materials with high in vitro protein digestibility always had low in vitro starch digestibility.

Kumar and Venkataraman (1976) investigated the in vitro starch digestibility of cowpeas. They found values of 37 and 44%, respectively, for uncooked and cooked whole seeds; and 39 and 90%, respectively, for starch isolated from uncooked and cooked seeds. Jyothi and Reddy (1981) reported in vitro starch digestibility values of 52% for raw cowpeas and 58% for those boiled for 30 min. They concluded that cooking before amylolysis considerably increased the digestibility. Their findings agree with the values for the control group, but not with those of the test group in the present study, in which the starch digestibility of raw flour was higher than that of cowpeas boiled for 45 and 90 min (Table

V). The in vitro starch digestibility of cowpeas reported by Nnanna and Phillips (1990) ranged from 40 to 95%. It was 90% for decorticated and cooked cowpeas (boiled for 1 hr), compared to 71 and 63% in the present study for the control group boiled for 90 and 45 min, respectively. It is possible that the dry grinding used by Nnanna and Phillips (1990) made cell contents more available to enzyme reaction.

In the present study, no starch was detected in feces samples. This was possibly due to the conversion of low levels of starch to a resistant type by autoclaving (French 1984); however, a comparison of autoclaving and gelatinization by KOH treatment revealed little difference. It is more likely that any starch escaping from the ileum was fermented by colonic bacteria, producing an apparent digestibility of 100%. Nevertheless, in order to avoid the possibility of generating resistant starch, a modified method using 4*N* KOH instead of autoclaving for gelatinization was applied to determine the starch content in the ileum.

Results of the effect of storage and processing on ileal starch digestibility are shown in Table IV. It is interesting that neither the storage conditions nor the processing treatment alone had an effect on ileal starch digestibility, despite the differences observed with the in vitro method. The test extrudates and the control flour had the lowest digestibilities among all samples; they were significantly different from the extruded control sample (Table V).

No relationship was observed between the values for in vitro and ileal starch digestibility. The in vitro digestibility ranged from 44 to 94%, and the ileal digestibility from 84 to 97%. The large difference between the two methods may be attributed to the ability of animals to break open intact cells and digest their contents better than in vitro digestion using two enzymes. This would explain the particularly low in vitro digestibility of boiled cowpea seeds that were wet-milled in an attempt to simulate chewing. Gentle disruption of cooked seeds is known to result in the separation of whole cells in control seeds and clumps of HTC seeds. The in vitro digestibility of raw flour starch was probably limited by both intact starch granules and enzyme inhibitors. Nevertheless, poor correlation was observed between starch digestibility and overall feed efficiency determined in a subsequent study (Tuan and Phillips, *unpublished*) indicating that starch digestibility has little influence on overall nutritional quality. Paredes-Lopez et al (1989) found no difference in starch gelatinization temperature between whole seeds and isolated starch from fresh and hard beans. Hohlberg and Stanley (1987) found that the gelatinization temperature increased with storage at either low or high temperatures and relative humidities, but they found no significant differences due to the combined effects of storage conditions and hardening.

CONCLUSIONS

Storage of cowpeas under conditions that produced the HTC defect reduced protein digestibility as determined by both an in vitro technique and analysis of rat ileal contents. True (fecal) digestibility in the rat detected no difference due to storage. Among processing treatments, boiling whole seeds for 45 min produced the highest digestibilities, and extrusion cooking produced the lowest. Raw flour was poorly digested, as expected, probably because of both active antinutritional factors and effects of HTC development. There was a significant interaction between the storage conditions and the processing treatment. Contrary to what had been expected, the effects of HTC development were most pronounced when cowpeas were ground before cooking, and the high-shear environment of the extruder produced the greatest effect. It had been hypothesized that disrupting the aggregated, rigid cells of HTC seeds before cooking would allow more efficient access of water during cooking and proteolytic enzymes during digestion. The observed exacerbation of the negative effect of HTC development on protein digestibility may have been due to interaction between proteins and phenolic acids. The latter are mobilized in the cell wall during development of the defect (Srisuma et al 1989). Storage at high temperature and relative humidity has been shown to result in an increase in endogenous protease activity and a consequent hydrolysis of bean storage protein (Hohlberg and Stanley 1987). This might be expected to increase overall protein digestibility unless it also resulted in increased interactions with digestibility-limiting agents.

In contrast to protein digestibility, starch digestibility exhibited little effect from the development of the HTC defect. Although differences were significant only in the case of extruded flour, the trend of starch digestibility was generally opposite that of protein digestibility, that is, starch from HTC and extruded seeds was more digestible than that of control and whole boiled seeds. The effects of processing on starch digestibility could be explained by the disruption of granules, as in our original hypothesis mentioned above. Slight positive effects of storage on starch digestibility could have been due to the action of endogenous amylolytic enzymes activated, as phytase and pectin methylesterase are thought to be, by high temperature and humidity in storage.

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