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Protein Nutritional Quality of Traditional and Novel Cowpea Products Measured by *In Vivo* and *In Vitro* Methods

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ABSTRACT

Effect of processing on nutritional quality of cowpea meal protein was determined by *in vivo* and *in vitro* methods. Uncooked meal and meal which had been extruded at various temperatures and moistures; slurried, steamed, and drum-dried (SDP); and hydrated to a paste and deep-fat fried (akara) were studied. PER values of extrudates (1.81–1.97), and akara (1.89) were higher than those of raw meal (1.44) or steamed, drum-dried paste (1.63). The saturation kinetics model showed similar trends but differences were not significant. *In vitro* digestibility was highest for extrudates (83–85%), intermediate for akara and SDP (82.8%, 81.2%) and lowest (77.8%) for raw meal. C-PER/DC-PER gave differing and contradictory results.

INTRODUCTION

COWPEAS are an important source of protein in developing countries, especially in West Africa where they are eaten in a variety of ways (Dovlo et al., 1976). Like other legumes, cowpeas contribute to the level of dietary protein in starchy tuber-based diets through their relatively high protein content 25%, and to the quality of dietary protein by forming complementary mixtures with staple cereals. However, even in locations where they are widely consumed, but especially in industrialized countries, starchy legumes are underutilized because of their low sociocultural status. This lack of popularity is due to deficiencies in appropriate technology for producing acceptable traditional and novel food products and because of reservations about nutritional quality, especially digestibility.

A number of studies have been directed toward improving traditional cowpea products and processes for making them (Adeniji and Potter, 1980), including a major effort in this department (McWatters, 1983; McWatters and Chhinnan, 1985). Likewise, research has been applied to developing new food ingredients and products made from cowpeas and other starchy legumes (Zamora and Fields, 1979; Phillips, 1982a; Sosulski et al., 1982). Extrusion cooking is one of the most versatile and efficient techniques for producing ingredients and novel foods (Harper, 1981), although its application to starchy legumes has been limited. Several authors have studied the effects of extrusion on either nutritive quality (Elias et al., 1976; Jorge Jao et al., 1980) or on texture (Jeunink and Cheftel, 1979) of grain legumes including cowpea. However, with few exceptions (Fham and del Rosario, 1984a,b), studies on both textural and nutritional properties of the same products have not been presented.

Recently an investigation of the extrusion cooking of cowpea meal under a range of feed moisture and barrel temperature conditions which lead to products with a variety of physicochemical and textural characteristics has been reported (Phillips et al., 1984; Kennedy et al., 1986). Results from a subsequent study of protein nutritional quality of these extrudates, a steam-cooked, drum-dried slurry, the traditional deep-fat-fried paste, akara, and the meal from which all products were made are presented in this report. A concomitant objective was to compare several methods for measuring protein nutritional quality as they applied to these products.

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MATERIALS & METHODS

Processing cowpeas

Mississippi silver-hull crowder cultivar cowpeas (*Vigna unguiculata*) were obtained from Pennington Seed Company, Madison, GA. Seeds were decorticated by cracking and aspiration as described by Phillips (1982a). Decorticated seeds were ground to a meal on a centrifugal mill equipped with a screen having 1.0 mm perforations (Retch GmbH, Haan, West Germany, Model ZM1).

Slurries of cowpea meal in water (1 kg in 2.4 L) were heated in a steam kettle with constant stirring to a temperature of 80°C. The gelatinized paste was diluted with water to approximately 5% solids and dried on a 15.24 x 20.32 cm drum drier (Model ALC-4, Blaw Knox Food and Chemical Equipment Co., Buffalo, NY) at 70 psi steam pressure (158°C surface temperature).

Akara was made according to the method of McWatters (1983). Batches of 280 g cowpea meal were mixed with 322 g tap water for 5 min at low speed (#1), then whipped for 90 sec at the high speed (#3) in a Hobart mixer (Model M-50, Hobart Mfg. Co. Troy OH). The resulting stiff foam was formed into balls and fried in peanut oil at 204°C for 60 sec per side in a Belshaw Automatic Cutter/Continuous Fryer (Model D MM110, Belshaw Brothers, Inc., Seattle, WA). Fried akara balls were frozen, freeze dried (Vacudyne Corp., Chicago, IL), and defatted by repeated extraction with petroleum ether for 24 hr at 25°C. After 24 hr desolventization in a stream of room temperature air, defatted akara balls were ground to a meal using the conditions described above.

Cowpea seeds which had been decorticated as described above were hydrated to total moisture of 20, 30 and 40% and chopped to coarse meals as described by Phillips et al. (1984). These meals were extruded at barrel temperatures of 150, 175, and 200°C in a Wayne pilot scale (19 x 475 mm barrel) extruder (Wayne Machine and Die Co., Totowa, NJ) as described by Kennedy et al. (1986). The nine extrudates were freeze-dried as described for akara. Extrudates are designated according to the conditions under which they were produced. For example, "E20-150" represents the extrudate produced from 20% moisture meal at a nominal barrel temperature (Phillips et al., 1984) of 150°C.

Cowpea products were assayed for protein by Kjeldahl analysis and for fat by extraction for 24 hrs with petroleum ether in a Goldfisch apparatus.

In vivo protein quality determination

Protein nutritional quality was estimated *in vivo* by feeding diets containing graded levels of cowpea products to weanling, male Sprague-Dawley rats for 21 days and measuring protein consumed (intake) and change in body weight (response). The design allowed both linear and nonlinear models to be applied to the data. Animals were individually housed in stainless or galvanized steel cages in an environmentally controlled room (20°C, alternate 12-hr light/dark periods). Individuals, which were distributed into groups of 5 and assigned particular diets according to the procedure of Hackler (1978), received diet and water ad libitum. Feed intake was determined on alternate days and weight change, once per week.

Isocaloric diets (Table 1) were prepared from casein (standard diet); unheated cowpea meal; steamed, drum-dried paste (SDP); defatted akara; and extrudates, E20-150, E30-150, E20-175, E40-175 and E30-200. Constraints on the number of animals that could be accommo-

Table 1—Composition of Diets

Component	Percentage of diet
Protein	2. 5, 10, 13 or 17%
Soybean Oil	8%
Vitamin Diet Fortification Mix[a]	2.2%
William Briggs Modified Mineral Mix[b]	3.5%
Corn Starch	82.3, 79.3, 74.3, 71.3, or 67.3% (varied with respect to protein concentration)

[a] ICN vitamin fortification mix (Anon., 1980). Contains (g kg): vitamin A (200,000 units g), 4 5; vitamin D (500,000 units g), 0.20; α-tocopherol, 5.0; ascorbic acid, 45: inositol (d_2H_2O), 5.0; choline chloride, 75; menadione, 2.25; p-aminobenzoic acid, 5.0; niacin, 4.5; riboflavin, 1.0; pyridoxine, 1.0; thiamine, 1.0; Ca pantothenate, 3.0; biotin (mg kg), 20.0; folic acid (mg kg), 90.0; and vitamin B_{12} (0.1% in gelatin, g kg), 846.09.

[b] Cohen et al. (1967). Contains (g kg): $CaCO_3$, 207.14; $CaHPO_4$, 322,85; $CuSO_4$ (100 mesh), 0.37; $MgSO_4$, 65.71; $MnSO_4 \cdot H_2O$, 4.40; KCl (40 mesh), 208.57; KIO_3, 0.02; Na_2HPO_4 (40 mesh), 186; $ZnCO_3$, 0.6; and ferric citrate (16.7% Fe), 4.31.

Table 2—PER and corrected [a] PER for cowpea products and standard casein

Protein source	Mean PER	Mean corrected PER
Casein	3.54[a]	2.50
Raw	2.04[d]	1.44
SDP	2.31[cd]	1.63
Akara	2.68[b]	1.89
E20-150[f]	2.76[b]	1.95
E20-175	2.65[b]	1.86
E30-150	2.81[b]	1.97
E30-200	2.57[bc]	1.81
E40-175	2.80[b]	1.97

[a] Values corrected to $PER_{casein} = 2.50$ by multiplying by 2.50/3.54.

[b-e] Mean values not sharing the same superscript are significantly different at $P < 0.05$.

[f] E20-150 refers to extruded cowpea meal produced at 20% feed moisture and 150 C barrel temperature. Other designations follow the same pattern.

dated prevented examination of all extrudates. In addition, a group of 5 animals received a protein-free diet.

Data from the feeding study were utilized to estimate protein quality using the protein efficiency ratio (PER) and the modified saturation kinetics (SK) models (Phillips, 1982b). The SK model (Flodin et al., 1977) has the form: $r = (bK_I + R_{max} I^n)/(K_I + I^n)$ where r is response and I is intake. The parameter b is the intercept, R_{max}, the asymptotic value of r when I is very large, K_I, a nutrition constant, and n, the apparent kinetic order. For the modified model, n was assigned a value of −11g and R_{max} a value of 165g. Quality was estimated as intake required to produce specified responses, $I = (K_I(r - b)/(R - r))^{1/n}$; at maintenance (r = 0), half-maximal ($r = 0.5(R_{max} - b) + b$), and 95% maximal ($r = 0.95(R_{max} - b) + b$) response. Computation, curve-fitting and statistical analyses were performed on an IBM 4361 computer using the Statistical Analysis System (SAS) package (Helwig et al., 1976).

In vitro protein quality determination

In vitro protein quality was determined for unprocessed cowpea meal; steamed, drum-dried paste (SDP); defatted akara; and all nine extrudates. Amino acid analysis was performed by ion exchange chromatography on a Durrum D-500 analyzer (Dionex, Inc., Sunnyvale, CA) using the manufacturer's directions following sample preparation as described by Phillips (1983). *In vitro* digestibility (IVD) and computed PER (C-PER) and discriminant-computed PER (DC-PER) were determined by the methods of Satterlee et al. (1982). IVD was calculated from the change in pH of protein samples digested with solutions of trypsin, chymotrypsin, porcine intestinal peptidase and bacterial protease after 20 min. C-PER was computed from the essential amino acid profile corrected by *in vitro* digestibility using a series of discriminant equations. DC-PER was calculated solely from amino acid profile using other discriminant equations which also provide an estimate of digestibility. Calculations of computed PERs were done using a computer program supplied by L. D. Satterlee.

RESULTS & DISCUSSION

COWPEAS resemble other legumes in their potential contribution to protein nutrition based on amino acid profile. In addition they are lower in antinutritional factors than many other legumes as reflected by moderately high PER values of even unheated seed (Phillips and Adams, 1982) Nevertheless, careful processing has been found to improve protein quality (Elias et al., 1976). Because of the constraints on consumption of starchy legumes in both developing and industrialized countries, it is important to assess the effect of both traditional and contemporary processing techniques on nutritional quality. In addition, this study provided the opportunity to compare several *in vivo* and *in vitro* techniques for measuring protein quality.

Results of the PER assay are presented in Table 2. There is considerable variation in PER of cowpeas in the literature (Phillips and Adams, 1983), but the value for unheated, decorticated meal found in this study was in the range reported by other investigators. All processes except steaming-drum drying significantly improved PER of cowpea meal. It is not possible to determine from these data whether SDP is under- or overprocessed. Onayemi and Potter (1976) observed that drum drying a slurry of raw cowpea flour at 45 psi with minimal contact time improved PER from 1.34 to 1.64. Acton et al. (1983) reported that briefly heating a slurry of peanut meal to 121°C then drum drying improved PER from 1.43 to 1.62, but additional processing further improved quality. On the other hand, it has been shown that trypsin inhibitors in cowpea meal are destroyed very rapidly at moistures of 20% and temperatures of >125°C (Phillips et al., 1983), implying that they were no longer a factor in SDP. While there were no significant differences among extrudates, products made at lower temperatures and/or higher moistures generally tended toward higher values. Elias et al. (1976) examined various cooking methods for their effect on cowpea protein quality and found that the greatest improvement over the raw seed, an increase in PER from 1.21 to 1.73, occurred when the meal was extruded. Unfortunately, extrusion conditions were not specified. Extrusion improves protein digestibility and thus quality by denaturing proteins, including enzyme inhibitors and lectins, and reduces quality by promoting non-enzymatic browning when carbohydrate is present (Cheftel, 1986). Pham and del Rosario (1984b) reported that available lysine was reduced in extruded cowpea meal as moisture and temperature increased over the range 30–45% and 93–132°C. Since cowpea meal contains an excess of lysine, such losses must be severe to be detected by PER. Deep fat frying is obviously able to produce browning and loss of lysine at the surface of the food. However, a spherical product such as akara has a minimum surface to volume ratio and, at least as prepared under the conditions reported here, also had higher quality than raw meal.

The Saturation Kinetics (SK) assay is a nonlinear model which fits the entire range of possible nutrient intake-animal response data, and is thus able to predict quality at any intake or response (Flodin et al., 1977). However, it fails to converge to reasonable solutions and exhibits poor resolution when proteins of insufficient quality or concentration to produce the expected hyperbolic/sigmoidal intake-response pattern are studied. The two-parameter modification (Phillips, 1982b) was proposed to alleviate these problems. Both the original and the modified SK models were applied to data from this experiment. Results of the former are not presented because of the extremely large errors associated with parameter estimates. The modified model produced a good fit to the experimental data (Table 3), but resolution was still poor compared to PER. Based on overlap of confidence bounds, the equation for casein was different from those for all cowpea products, but none of the latter differed from each other. Nevertheless, quality estimates in terms of protein intake required to produce particular responses were calculated (Table 3) to allow general compar-

Table 3—Saturation kinetics parameters and quality estimates[a] when R_{max} = 165g and b = −11.0g

Protein Source	Equation parameters K (95% Confid. Bounds)	n (95% Confid. Bounds)	R^2	Quality estimates I_0	I_{05}	I_{095}
Casein	451 (33–869)	2.03 (1.75–2.32)	0.98	5.34	20.3	42.0
Raw meal	203 (97–308)	1.45 (0.07–1.30)	0.98	5.67	39.0	107
SDP	117 (78–156)	1.34 (1.25–1.44)	0.99	4.62	34.7	104
Akara	130 (76–185)	1.44 (1.32–1.56)	0.99	4.48	29.4	81.0
E20-150[b]	161 (42–279)	1.53 (1.32–1.74)	0.97	4.71	27.7	72.0
E20-175	109 (49–169)	1.40 (1.23–1.56)	0.98	3.87	29.6	89.0
E30-150	91 (50–131)	1.33 (1.20–1.46)	0.98	4.12	28.3	81.0
E30-200	93 (44–142)	1.29 (1.14–1.45)	0.98	3.94	28.0	81.0
E40-175	100 (40–159)	1.38 (1.20–1.55)	0.98	4.12	33.6	105

[a] Quality estimated as intake required to produce a specified response: I_0, zero response; I_{05}, half-maximal response; I_{095}, 95% maximal response.
[b] E20-150 refers to extruded cowpea meal produced at 20% feed moisture and 150°C barrel temperature. Other designations follow the same pattern.

ison to PER. This model predicted a higher required intake (and thus lower quality) for casein than most cowpea proteins to maintain body weight (r = 0). There is no obvious reason or such a phenomenon which was assumed to be an artifact of the method. At half maximal response, the model predicted from one third to twice more cowpea than casein should be required, and at 95% of maximal response, 2 to 2.5 times the amount of cowpea as casein was predicted to be required.

Amino acid content is the basis of potential nutritional quality and is required for the calculation of CP-PER/DC-PER. The profiles of cowpea products (Table 4) indicated no consistent effect of processing on amino acid content. This implied that any changes in quality were the result of changes in amino acid availability through increases in digestibility arising from denaturation of storage proteins and/or antinutritional factors. *In vitro* protein digestibility (Table 5) offered only partial support for this view. In contrast to SK, this method had very high resolution, being able to distinguish ~1% differences in digestibility. Raw meal and SDP which had the lowest quality by PER, and the implied lowest quality by SK also had the lowest IVD. In contrast, Akara which had the next lowest IVD exhibited intermediate quality by in vivo methods.

The C-PER model is based on amino acid profiles and *in vitro* digestibility, while in the DC-PER model digestibility and the final "PER" value are computed solely from amino acid content. For this reason, the former is more likely to detect the presence of residual antinutritional factors or damage from overprocessing. C-PER values (Table 5) lay within the range of rat PERs but the span was much smaller and the order was entirely different. DC-PER values were higher than C-PERs, more closely matched the range of rat values, and were more widely dispersed implying improved resolution. However, the order of quality was often reversed compared to the other methods. This reflects the inability of DC-PER to detect availability factors not related to amino acid profile.

Figure 1 summarizes the results of applying *in vivo* and *in vitro* methods to cowpea products by arranging the scores on a relative scale where casein is the standard with a score of 100. It is obvious that relative quality of cowpea products as a group varied widely with the specific assay, and further, ordering within the group exhibited method-to-method variation. PER, although it features the false assumption of zero intercept at zero protein intake and provides a only a single measure of quality which is valid at suboptimal protein intakes, demonstrated higher resolution than the saturation kinetics model. The latter method was unable to distinguish differences between even the extreme cowpea products, yet it clearly showed how absolute and relative quality varies with specified response. Cowpea products were predicted to be equivalent to casein at low response, but rapidly fell behind as demand for growth increased. The obverse is that cowpea protein could produce the same response as casein if intake were sufficiently increased, and that the required intake may be predicted by the SK model. It is unfortunate that statistical limitations on this method compromise its utility, since it overcomes so many shortcomings of traditional linear methods (Flodin et al., 1977; Phillips, 1982b). Of the *in vitro* methods, IVD is most firmly rooted in physiological fact. However, Bodwell et al. (1980) found poor correlation between IVD and digestion in humans and rats for a range of products, but observed that correlations improved if samples from similar sources were studied. The C-PER and DC-PER models, while they occasionally predicted values similar to those from the rat, generally varied so much

Table 4—Essential amino acid content of cowpea products as compared to the FAO/WHO reference profile

E.A.A.	Range (g/16g N) Cowpea treatments (95% recovery)	Mean (g/16g N) Cowpea treatments (95% recovery)	FAO reference profile
LYS	7.31– 7.73	7.55	5.5
MET & CYS	2.22– 2.49	2.40	3.5
THR	3.51– 3.84	3.68	4.0
ILE	3.70– 4.42	4.33	4.0
LEU	8.62– 8.96	8.75	7.0
VAL	4.99– 5.12	5.02	5.0
PHE & TYR	9.93–10.28	10.07	6.0
TRP	1.54– 1.74	1.59	1.0

Table 5—In vitro and predicted protein digestibility, C-PER, and DC-PER values for cowpea products and standard casein

Treatment	*In vitro* Digestibility	C-PER	Predicted Digestibility	DC-PER
Raw	77.82[h]	1.68	92.95	1.96
Drum Dried	81.21[g]	1.65	92.89	1.89
Akara	82.84[f]	1.66	91.11	1.90
E20-150[i]	84.80[b]	1.74	90.81	1.86
E20-175	84.42[bc]	1.69	92.82	1.86
E20-200	84.53[bc]	1.72	91.73	1.86
E30-150	83.69[de]	1.74	92.30	1.90
E30-175	83.97[cd]	1.75	88.78	1.87
E30-200	83.30[ef]	1.63	90.75	1.78
E40-150	84.31[bcd]	1.62	90.47	1.73
E40-175	84.31[bcd]	1.76	90.07	1.88
E40-200	84.25[bcd]	1.71	91.19	1.85
Casein	89.80[a]	[2.50]	90.00	[2.50]

[a-h] Mean values not sharing the same superscript are significantly different at P ≤ 0.05.
[i] E20-150 refers to extruded cowpea meal produced at 20% feed moisture and 150°C barrel temperature. Other designations follow the same pattern.

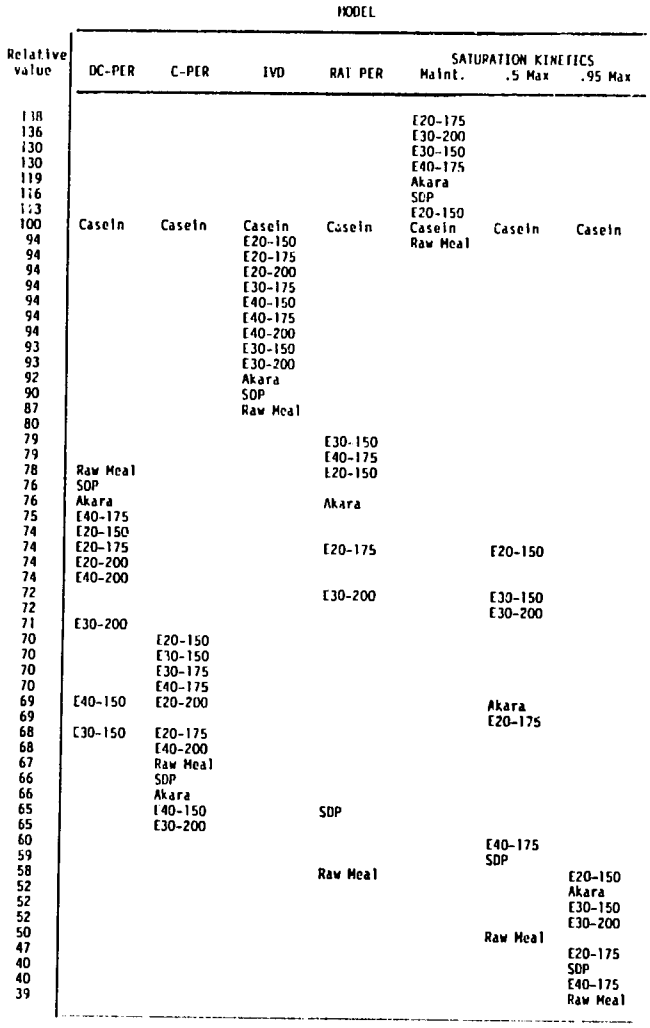

Fig. 1—Relative nutritional quality estimates (Casein 100) for cowpea materials as determined by the different models. Note that scale is not linear.

both in order and in magnitude as to be of very questionable utility.

Based on the various methods used to assess protein quality of cowpea products in this study, it seems reasonable to conclude that although the exact order of quality varied according to method, both the traditional process for making akara and contemporary techniques such as extrusion are able to produce nutritious as well as texturally varied products. Further research on factors responsible for desirable sensory properties of extruded cowpea products will be necessary before acceptable novel foods can be produced.

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