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The effect of heat treatment on the in-vitro multienzyme digestibility of protein of six varieties of African yam bean (*Sphenostylis stenocarpa*) flour

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The in-vitro multienzyme protein digestibilities of the flours of six colour varieties of African yam bean (*Sphenostylis stenocarpa*), made from both hulled and dehulled seeds were investigated. The multienzyme system consisted of trypsin, chymotrypsin and peptidase. Digestibilities were determined for a 15 min digestion period. Both dehulling and heat treatment improved digestibility. Comparison of flours from raw hulled seeds with those from raw dehulled seeds showed that digestibility was 7% better in the latter. Heat-treated hulled-seed flours gave a digestibility increase of 6% compared with raw flours whereas in the dehulled samples the digestibility increase of heat-treated flours over raw was 5%. Heat-treated dehulled seeds were better, with a digestibility increase of 6% over the heat-treated hulled seed flours. No significant differences ($P < 0.05$) were observed in the digestibilities among the whole-seed flours but significant differences were observed in the dehulled-seed flours. © 1997 Elsevier Science Ltd

INTRODUCTION

The African yam bean (AYB: *Sphenostylis stenocarpa*) belongs to the family of Papilionaseae which is sometimes classified as the sub-family Leguminosae (Okigbo, 1973). It is usually cultivated for its edible seeds and tuberous roots. The AYB is cultivated in parts of Africa (in Central African Republic, Gabon, Zaire and Ethiopia) for its potato-like spindle-shaped tubers or for its edible seeds (in Nigeria) (Dalziel, 1955). Highest seed yields are obtained in mixed plantings with yams, maize, okra and other vegetables (Phillips, 1972).

The AYB is one of the under-utilised legumes cultivated in Nigeria (Aletor & Aladetimi, 1989). AYB and lima bean (*Phaseolus lunatus*) have both been cited as legumes having exceptional potential for adaption to lowland tropical conditions and as potentially important food legumes (Rachie, 1972).

The characteristic problem of being hard to cook which hinders the extensive use of AYB has been substantially reduced by precooking treatments (Njoku *et al.*, 1989). Studies have been carried out on the in-vitro multienzyme digestibility of protein of AYB (Oshodi & Hall, 1993; Oshodi *et al.*, 1995), amino acid composition of AYB flour (Oshodi *et al.*, 1995) and functional properties of some varieties of AYB (Adeyeye *et al.*, 1994).

Digestibility of protein and bioavailability of its constituent amino acids are very important factors in determining protein quality (Hsu *et al.*, 1977; FAO/WHO, 1990; Suman *et al.*, 1992). This is true because not all proteins are digested, absorbed and utilised to the same extent (FAO/WHO, 1990). Differences in protein digestibility may arise from inherent differences in the nature of food protein, from the presence of non-protein constituents which may modify digestion, from the presence of anti-physiological factors or from processing conditions that alter the release of amino acids from proteins by enzymatic processes (FAO/WHO, 1990).

The development of useful in-vitro methods for the determination of protein digestibility has been reported (FAO/WHO, 1990). In particular the multienzyne in-vitro procedure has shown good correlations with in-vivo methods (Hsu *et al.*, 1977; Pedersen & Eggum, 1983; FAO/WHO, 1990). This paper reports on the in-vitro multienzyme protein digestibility of AYB (hulled/dehulled) seed flours when raw and when heat-treated.

MATERIALS AND METHODS

African yam bean (*Sphenostylis stenocarpa* Hochst ex A Rich) seeds were collected from the farm located at

Ayedun-Ekiti, Ondo State, Nigeria. Six different colour varieties of white (A_1), light-brown with black strips (B_1), reddish-brown (C_1), reddish-brown with black strips (D_1), light-brown (E_1), and black with light-brown strips (F_1) were identified, sorted and screened to remove the bad seeds. Their corresponding dehulled samples labelled A_2, B_2, C_2, D_2, E_2, and F_2 were also processed for analysis. The dried mature seeds and dehulled samples were dry-milled into fine flours.

The removal of the testa and the preparation of the dehulled samples were carried out according to the method of Oshodi and Ekperigin (1989).

The proximate analysis of the samples for moisture, ether extract, total ash and crude fibre were carried out at least in triplicate using the methods described by AOAC (1990). Nitrogen was determined by the micro-Kjeldahl method described by Pearson (1976) and the nitrogen content was converted to protein by multiplying by 6.25.

The determination of in-vitro protein digestibility was carried out using the method of Hsu *et al.* (1977). Fifty millilitres of an aqueous suspension of the sample (6.25 mg sample per ml) in distilled water was adjusted to pH 8.0 with 0.1 M HCl and/or 0.1 M NaOH, while stirring in a 37°C water bath. The multienzyme solution consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase per ml was maintained in an ice bath and adjusted to pH 8.0 with 0.1 MHCl and/or 0.1 M NaOH. Class-distilled water was used in preparing all solutions. The enzymes were purchased from Sigma Chemical Co (St Louis, MO, USA). A 5 ml sample of the multienzyme solution was added to the sample suspension with constant stirring at 37°C. The pH of the suspension was recorded 15 min after the addition of the multienzyme solution and the in-vitro digestibility was calculated using the regression equation of Hsu *et al.* (1977):

$$Y = 210.46 - 18.10X$$

Table 1. Proximate composition (g/100 g) of the African yam bean flour (hulled seeds)

Bean Variety[a]	Moisture	Crude protein	Ether extract	Total ash	Crude fibre
A_1	9.80	20.5	5.76	3.80	5.01
B_1	7.29	20.3	9.53	2.99	5.66
C_1	7.18	20.5	10.18	2.91	5.42
D_1	8.85	21.1	17.89	2.40	6.49
E_1	7.74	19.3	10.74	2.89	5.20
F_1	8.48	21.1	7.98	2.51	6.57
Mean	8.22	20.5	10.35	2.92	5.73
SD[b]	1.01	0.66	4.11	0.49	0.66
CV (%)[c]	12.29	3.23	10.04	16.93	11.54

[a]A_1 (White); B_1 (light brown with black strips); C_1 (reddish brown); D_1 (reddish brown with black strips); E_1 (light brown); F_1 (black with light brown strips).
[b]Standard deviation.
[c]Coefficient of variation.

where Y is in-vitro digestibility (%), X is the pH of the sample suspension after 15 min digestion with the multienzyme solution.

Sample suspensions were also prepared as above, heated to boiling point and allowed to boil for 15 min as described by Grant *et al.* (1983), cooled and incubated at 37°C. The digestibilities of these heat-treated samples were determined using the multienzyme solution as described above. The multienzyme solution was freshly prepared before each series of tests and the enzyme activity was determined by using a casein of known in-vivo apparent digestibility (Hsu *et al.*, 1977). All chemicals used were of analytical grade. All the data generated were analysed statistically (Steel & Torrie, 1960).

RESULTS AND DISCUSSION

The proximate composition of the samples varied, depending on the varieties. For example, the crude protein varied from 19 g/100 g in E_1 to 21 g/100 g in F_1, ether extract varied from 6 g/100 g in A_1 to 18 g/100 g in D_1, total ash varied from 2 g/100 g in D_1 to 4 g/100 g in A_1, crude fibre varied from 5 g/100 g in A_1 to 7 g/100 g in F_1 and moisture varied from 7 g/100 g in C_1 to 10 g/100 g in A_1. The intervarietal similarity of the various parameters is attested to by the low coefficients of variation with the least variation (3.23%) shown in protein. The ether extract of D_1 appeared very high when compared to other varieties. This difference may be due to genotype since the growth conditions were similar for all the varieties and the method of extraction was also similar for all the samples.

The proximate composition of the corresponding dehulled seeds also depends on the variety. For example, the crude protein varied from 20 g/100 g in C_2 to 26 g/100 g in E_2, ether extract varied from 2 g/100 g in D_2 to 10 g/100 g in C_2 and moisture varied from 3 g/100 g in E_2 to 7 g/100 g in A_2. The major differences for moisture and ether extract are easily seen in the higher coefficients of variation shown in Table 2. Tables 1 and 2 show that the dehulled samples are more concentrated sources of protein whereas the hulled samples are better sources of ether extract and (likely) mineral elements. Since the varieties were all grown under the same conditions and the same analytical methods were used, the differences in values can only be due to genetic variations in the samples.

Table 3 illustrates the in-vitro protein digestibilities of the raw and heat-treated hulled seed samples. The average digestibility value of raw hulled seeds was 73% with a coefficient of variation of 0.86% while the corresponding heat-treated samples gave an average value of 79% with a coefficient of variation of 1.33%. The low coefficients of variation in the table indicate that the direction and rate of change in digestibility is not dependent on variety. These results follow the trend of

Table 2. Proximate composition (g/100 g) of the African yam bean flour (dehulled seeds)

Bean Variety[a]	Moisture	Crude protein	Ether extract	Total ash	Crude fibre
A_2	7.10	22.5	8.00	2.31	1.61
B_2	6.27	23.0	7.64	2.09	2.38
C_2	5.65	20.2	10.18	2.20	1.88
D_2	7.01	23.7	1.93	2.36	1.96
E_2	3.20	25.8	3.60	2.06	2.02
F_2	6.64	22.3	5.41	2.32	2.22
Mean	5.98	22.9	5.21	2.22	2.01
SD	1.46	1.83	2.34	0.13	0.27
CV (%)	24.41	8.00	44.98	5.86	13.35

[a]A_2, B_2, C_2, D_2, E_2, F_2, are dehulled samples of A_1, B_1, C_1, D_1, E_1, F_1, respectively, as in Table 1.

Table 4. In-vitro protein digestibility of African yam bean flour (dehulled seeds)

	% Digestibility		
Sample[a]	Raw samples	Heat-treated samples	Difference
A_2	77.5	82.9	5.43
B_2	76.3	81.8	5.52
C_2	82.3	84.7	2.44
D_2	83.2	89.3	6.07
E_2	82.9	88.6	5.70
F_2	77.5	83.1	5.61
Mean	80.0	85.1	5.13
SD	2.89	2.86	1.34
CV (%)	3.61	3.36	26.03

[a]See footnote *a* to Table 2.

the report of Oshodi *et al.* (1995) but with slightly higher values for the digestibility and lower values for the coefficients of variation.

Table 4 illustrates the in-vitro protein digestibilities of the raw and heat-treated dehulled samples. The average digestibility value of raw dehulled samples was 80% while the corresponding heat-treated samples gave an average value of 85%. The coefficients of variation (3.61 and 3.36%, respectively) were also low but higher than those of the hulled seeds. Also, these values are correspondingly higher than the values reported by Oshodi *et al.* (1995) although the variations are correspondingly higher as well. It should, however, be noted that, while these experiments were carried out at a 15-min digestion period, those of Oshodi *et al.* (1995) were carried out at 10 min.

Tables 3 and 4 have columns under differences. Table 3 shows that the differences in the digestibilities between raw and heat-treated hulled samples ranged between 5 and 7% with an average of 6% and a variation of 12.9%. This shows that heat-treatment improves digestibility. Table 4 also shows digestibility differences of heat-treated dehulled seeds over raw seeds with values ranging between 2 and 6% with an average value of 5% and a variation of 26.0%. A critical look at Table 4 containing dehulled samples, columns two and three, shows that the digestibilities of the raw samples and the heat-treated samples may each be categorised into two sets based on the nearness of the results. In the raw samples, set one is A_2, B_2 and F_2 while set two is C_2, D_2 and E_2. When the two sets were subjected to student's t-test, a significant difference ($P < 0.05$) was found between the two sets. In the heat-treated samples, set one is A_2, B_2 and F_2 while set two is C_2, D_2 and E_2. On subjecting them to student's *t*-test, a significant difference was also found. These significant differences may be due to the unequal distribution of the seed hull which was now absent from the samples. The report of Oshodi *et al.* (1995) only showed significant difference in the heat-treated dehulled samples. The significant difference in the raw dehulled samples here may be due to the longer digestion period.

Table 3. In-vitro protein digestibility of African yam bean flour (hulled seeds)

	% Digestibility[b]		
Sample[a]	Raw samples	Heat-treated samples	Difference
A_1	74.1	81.2	7.15
B_1	72.3	78.2	5.97
C_1	73.4	78.2	4.80
D_1	73.0	79.6	6.61
E_1	72.5	79.0	6.52
F_1	72.4	78.5	6.06
Mean	72.9	79.1	6.19
SD	0.63	1.05	0.80
CV (%)	0.86	1.33	12.93

[a]See footnote *a* to Table 1.
[b]The digestibility for casein is 94.3%.

Heat-treated flours were better digested in all cases. Wallace *et al.* (1981) have reported that heat treatment of legume proteins and protein-containing flours improves digestibility; Phillips *et al.* (1983) made a similar observation with cowpea flour. Heating improves digestibility due to protein denaturation which results in opening of protein structure (Elias *et al.*, 1976; Sathe *et al.*, 1982; Grant *et al.*, 1983); heating also destroys protease inhibitors (Osborne & Mendel, 1917; Linener, 1983). Both denaturation and destruction of protease inhibitors cause easier hydrolysis by proteases.

The digestibilities of raw hulled seeds/dehulled seeds after 15 min digestion were compared. The digestibility of raw dehulled seeds was better than that of the hulled seeds by an average of 7%. This difference is bigger than the result of Oshodi *et al.* (1995) who carried out their digestibilities at 10 min. Also, the digestibility of heat-treated samples of hulled seeds and dehulled seeds was compared after 15 min digestion. In-vitro protein digestibility in the dehulled seeds was better than that of the hulled seeds by an average of 6% which is better than the result of Oshodi *et al.* (1995). This means that

dehulling the seeds improved the digestibility in all cases.

Heat treatment also improved the digestibility of both hulled and dehulled seeds. The negative effects of cooking on tannins will enhance digestibility, bioavailability and utilization of the proteins of AYB (Munro & Bassir, 1969). These results are in agreement with the observations of Hsu *et al.* (1977); Grant *et al.* (1983) and Abbey and Berezi (1988) on heat-treated legumes. The current results are slightly lower than the results for pigeon pea which is 77% for raw samples and 84% for heat-treated samples (Oshodi & Hall, 1993) when compared with the AYB hulled-seed flours. Tables 3 and 4 show that both dehulling and heat-treatment improved the digestibilities of the AYB flours. The improvement in the performance of rats fed AYB which have been cooked for a long time and those fed dehulled moist heat-treated bean flour, over rats fed dehulled dry heated flour, suggests that a long cooking time and moist heat treatment have beneficial effects related to the destruction of trypsin inhibitors and other anti-nutrients (Onayemi *et al.*, 1976; Abbey & Berezi, 1988).

CONCLUSION

From the current report, the dehulling of the seeds, the longer digestion period and the longer moist heat treatment all contributed to higher protein digestibility of the AYB.

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