Nutritional Quality of Hydrogen Peroxide Treated Groundnut Protein

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Treatment of groundnut protein dispersion with hydrogen peroxide to destroy aflatoxin impaired the protein quality of isolated protein. The NPU and PER values of the treated isolate were significantly lower than those of the untreated isolate. Supplementation of the H_2O_2-treated protein isolates with lysine, methionine, and threonine promoted growth in rats comparable with that of the untreated protein fed at 10% level. Similar growth response was obtained when the H_2O_2-treated protein isolate was fed at 20% level in the diet without any amino acid supplementation; however, the efficiency of protein utilization was lowered.

INTRODUCTION

Groundnut is the major oilseed crop of India and has a good potential as a source of supplementary protein in human foods. Processes for the isolation of protein from groundnut kernel and meal have been reviewed (Natarajan, 1980). Groundnut kernels, as well as the screw-pressed cake or defatted meal, may contain variable levels (50–2000 ppb) of aflatoxin arising from fungal infection of the nuts. During protein isolation nearly 60–65% of the aflatoxin in the meal elutes with the protein fraction. The observations that aflatoxins have hepatotoxic properties in a variety of experimental animals and that they are also carcinogenic, mutagenic, and teratogenic have given rise to obvious important questions concerning the risks to animal and human health (Barnes and Butler, 1964; Wogan and Newberne, 1967; Petit and Taber, 1968; Amla et al., 1971).

Chemical or heat treatment of groundnut meal and protein isolate helps to destroy the aflatoxin (Austwick and Ayerst, 1963; Pomeranz, 1964). Among the chemicals, hydrogen peroxide has been found effective for the preparation of aflatoxin-free protein isolate (Sreenivasamurthy et al., 1967). Food materials such as milk and milk products, fish and fish products, and liquid egg white have also been treated with H_2O_2 to improve their functional, nutritional, and storage qualities (Njaa, 1962; Fish and Mickelson, 1967; Schmidt et al., 1969; Snider and Cotterill, 1972; Cuq et al., 1978; Strange, 1984; Ihekoronye, 1987; Patel et al., 1989). However, in our earlier studies on groundnut protein, H_2O_2 treatment was found to have deleterious effects on sulfur amino acids and available lysine (Sreedhara and Subramanian, 1981).

The present study was carried out to determine the effects of H_2O_2 on the nutritional quality of groundnut protein, with reference to parameters such as protein efficiency ratio (PER) and net protein utilization (NPU).

EXPERIMENTAL PROCEDURES

Screw-pressed groundnut cake prepared from decuticled (skin-free) groundnut (peanut; *Arachis hypogaea* L.) kernels procured from a commercial oil milling unit at Coimbatore, India, was used. The cake was ground to a meal in a plate mill to pass through 44 mesh size and the meal used for protein isolation. Methods for the preparation of protein isolate and hydrogen peroxide treatment have been described earlier (Sreedhara and Subramanian, 1981).

Table I. Chemical Composition, Bound Fat, and Aflatoxin Content of Groundnut Meal and Protein Isolates (Untreated and Treated)

constituents	groundnut meal, %	groundnut protein isolates	
		untreated, %	H_2O_2-treated, %
moisture	4.9	5.1	5.0
total fat	8.8	5.0	5.2
free fat	7.9	1.3	1.4
bound fat	0.9	3.7	3.8
protein (N × 6.25)	46.9	93.5	91.9
crude fiber	2.9	0.6	0.7
ash	4.7	1.0	1.2
aflatoxin B_1, ppb	333	667	ND[a]

[a] ND, not detectable.

Chemical Analysis. The chemical composition of the various samples was determined by the standard AOCS (1980) and AOAC (1980) procedures. Aflatoxin content was determined according to the method of Pons et al. (1966). The data presented are the averages of three replicate values.

Bioassays of Protein Quality. Animal feeding tests were carried out by using Wistar strain albino rats to determine NPU, net protein ratio (NPR), and PER of the protein isolates.

Net Protein Utilization. Weanling rats weighing about 55 g were distributed to four comparable groups of 10 rats each by randomized block design (Robert and Torrie, 1960; Snedecor and Cochran, 1961). The animals were housed individually in cages.

The basal diet was similar to that used by Campbell (1963) and contained 77% cornstarch, 10% vegetable fat, 10% sugar, 2% salt mixture, and 1% vitaminized starch. The protein samples were incorporated in the diet at 10 or 20% protein levels replacing cornstarch. Dry diet was served in aluminum cups with a lid having a suitable opening in the center to let the rats reach the diet without wasting uneaten feed. Diet and water were given ad libitum. Records of diet consumed each day were maintained. The duration of the experiment was 10 days. At the end of the experimental period, the rats were weighed, anaesthetized with diethyl ether, and killed. The carcasses were kept in a freezer (−30 °C) for a few days after which time they were cut open and oven-dried at 110 °C. The dry weights were recorded, and then each carcass was separately powdered in a Waring blender to yield uniformly ground samples. The nitrogen contents of the samples were determined and the NPU, PER, and NPR values calculated (NAS–NRC, 1963).

Growth Studies and Protein Efficiency Ratio. Young weanling male rats 21–23 days old were allotted to 10 groups of 10 rats each by randomized block design and fed the different diets for a period of 28 days. The other experimental conditions were similar to those detailed under the NPU study. The effect of supplementation of groundnut protein isolate with methionine, threonine, and lysine at optimal levels on the protein quality

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Table II. Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) Values of Groundnut Protein Isolate (Untreated and H_2O_2-Treated)

group	source of protein[a]	initial body wt, g	final body wt, g	change in body wt, g	food intake, g	PER	NPR
A	skim milk powder	54.9	79.6	24.7	83	2.9	4.0
B	protein isolate, untreated	54.8	70.8	16.0	76	2.0	3.1
C	protein isolate, H_2O_2-treated	54.8	52.0	−2.8	43	−[b]	1.4
D	protein-free diet	54.8	46.0	−8.8	46	−	−

[a] Protein level in diets: A–C, 10%; D, 0.5%. 10 rats per group. [b] − means negative.

Table III. Net Protein Utilization (NPU) Values of Groundnut Protein Isolate (Untreated and H_2O_2-Treated) and Skim Milk Powder[a]

group	source of protein	body N of test group, g	body N of nonprotein group, g	N intake by nonprotein group, g	N intake by test group, g	NPU mean
A	skim milk powder	2.0	1.2	0.04	1.3	63.9
B	groundnut protein isolate, untreated	1.8	1.2	0.04	1.3	49.8
C	groundnut protein isolate, H_2O_2-treated	1.4	1.2	0.04	0.7	36.5

[a] Standard error for groups A–C, ±2.15. 18 degrees of freedom. Test of significance (Student's t-test): A ~ B, 14.1***; B ~ C, 13.3***, A ~ C, 27.6***; [***, very highly significant ($P < 0.001$)].

was also studied. Amino acid deficiencies were corrected, on the basis of chemical scores, by supplementation with 1.175 g of *dl*-methionine, 1.15 g of L-lysine monohydrochloride, and 1.262 g of *dl*-threonine for every 100 g of protein.

RESULTS AND DISCUSSION

The proximate chemical composition and aflatoxin content of groundnut cake and protein isolate prepared from it with and without H_2O_2 treatment are given in Table I. The cake had 46.9% protein and 8.8% fat. The protein contents of the untreated and H_2O_2-treated isolates were 93.5 and 91.9%, respectively; the fat contents of the samples were also comparable, being 1.3 and 1.4%, respectively. Bound fat (AOAC, 1980) contents of groundnut meal and protein isolates, untreated and treated, were found to be 0.9, 3.7, and 3.8%, respectively. It is evident from these results that H_2O_2 treatment did not alter the pattern of free and bound lipids in the isolates, though it has clearly shown increased binding of fat to protein during the isolation of protein. This experiment was done to assess whether any adverse effects would be caused, such as the development of oxidized flavors in the isolate as a result of H_2O_2 treatment. The H_2O_2-treated isolate was lighter in color compared to the control sample, and no off-odor was noticeable.

The groundnut cake contained 333 ppb of aflatoxin B_1, while the protein isolated from it contained twice as much (667 ppb). The isolate prepared from the H_2O_2-treated extract had no detectable amount of aflatoxin.

The chemical scores of the proteins in groundnut meal and the two protein isolates, calculated from their essential amino acid composition with egg protein as reference, indicated appreciable losses of sulfur amino acids (cystine and methionine), lysine, and threonine as a result of H_2O_2 treatment (Sreedhara and Subramanian, 1981).

Animal Experiments. The growth performance and diet consumption of rats and the NPR and PER values of proteins calculated for 10-day feeding experiments are given in Table II. Rats fed on the nitrogen-free diet lost 8.8 g of body weight during the period. A gain in body weight was observed in the groups fed on skim milk powder (SMP) and untreated protein isolate, the values being 24.7 and 16.0 g, respectively. Animals fed on the H_2O_2-treated protein isolate diet failed to grow and showed a loss in body weight of about 2.8 g. The food intake of the SMP-fed and untreated protein isolate fed groups were comparable, the diet consumption being 83.0 and 76.0 g, respectively. Animals fed on the H_2O_2-treated isolate diet and the nitrogen-free diet group, consumed 43 and 46 g of food, respectively. The NPR values of the SMP and protein isolate, untreated and treated, groups were 4.0, 3.1, and 1.4, respectively; the PER values for the SMP and protein isolate, untreated, groups were 2.9 and 2.0, respectively. The PER value of the H_2O_2-treated groundnut protein isolate could not be calculated since there was a loss in body weight of rats. Thus, it could be concluded that there is considerable impairment in protein quality of groundnut protein isolate as a result of H_2O_2 treatment.

Data on the average body nitrogen content of rats in different groups and the calculated NPU values of the proteins (Table III) provide further confirmatory evidence on the adverse effects of H_2O_2 treatment on protein. The body nitrogen content of the group fed treated protein isolate was much lower (1.4 g) compared to the group on untreated protein isolate (1.8 g). There was much higher retention of body nitrogen in the SMP-fed group, which gave a value of 2.0 g. The calculated NPU values of the SMP and protein isolate, untreated and treated, groups were 63.9, 49.8, and 36.5, respectively, the difference between any two of these samples being very highly significant. The values of 63.9 for SMP and 49.8 for untreated groundnut protein isolate compare well with the literature values (Subramanian et al., 1962).

Data on the gain in body weight of rats, diet consumption, and PER values of proteins in the 28-day feeding tests are given in Table IV. The PER value of 1.80 in group C of our study compares well with the reported literature values of 1.6–2.0 (Jones and Divine, 1944; Joseph et al., 1960). The average gain in weight of animals fed on the untreated protein isolate diet was 31.1 g with a diet intake of 214 g; the PER value was found to be 1.43. Thus, it is seen that, as a result of isolation of protein, there is a significant lowering in the nutritive value of groundnut protein.

The difference in response of the rats fed the two isolates, viz. untreated and H_2O_2 treated, was indeed striking. The animals fed on the treated isolate diet consumed much less food (175 g) comapred to those fed the untreated isolate (214 g) and gave a gain in weight of only 6.6 g compared to 31.1 g for the untreated isolate group. The corresponding PER values were 0.49 and 1.43, respectively, indicating significant damage to protein quality in the H_2O_2-treated isolate. Dollear et al. (1968) also noticed a reduction in PER value when the rats were fed with H_2O_2-treated groundnut meal. Rakesh et al. (1972) also observed

Table IV. Protein Efficiency Ratio (PER) of Groundnut Protein of Cake, Isolates (Untreated and H_2O_2-Treated) Fed at 10 and 20% Protein Levels Compared with Skim Milk Powder and Casein Fed at 10% Protein Level[a]

group	diet	protein level, %	initial body wt, g	gain in body wt, g	diet consumed, g	PER mean[b,d]
A	skim milk powder	10	37.4	62.3	252	2.51
B	casein	10	37.4	57.2	231	2.37
C	groundnut cake	10	37.4	41.8	229	1.80
D	protein isolate, untreated	10	37.3	31.1	214	1.43
E	protein isolate, H_2O_2-treated	10	37.3	6.6	175	0.49
F	protein isolate, untreated + SW[c]	10	37.4	48.3	233	2.10
G	protein isolate, H_2O_2-treated + SW	10	37.3	32.2	222	1.38
H	groundnut cake	20	37.2	72.2	234	1.61
I	protein isolate, untreated	20	37.4	62.3	236	1.30
J	protein isolate, H_2O_2-treated	20	37.5	30.3	205	0.74

[a] 10 rats per group, randomized block design; 4-week duration. [b] Standard error of the mean based on 62 degrees of freedom; ±0.106 for all groups except for group I, ±0.114 (nine observations). [c] SW: supplemented with 1.175 g of *dl*-methionine, 1.150 g of L-lysine hydrochloride, and 1.262 g of *dl*-threonine per 100 g of protein. [d] Test of significance (diet, average PER): E, 0.49, J, 0.74; I, 1.30, G, 1.38; D, 1.43; H, 1.61, C, 1.80; F, 2.10; B, 2.37, A. 2.51. Two rats in group E and one rat in group I died during the experimental period. Also, two rats in group E lost their weights, and hence PER values for the rats in these blocks getting other diets were also omitted. PER value corresponding to one rat which died in group I was estimated by the Missing Plot technique (Snedecor and Cochran, 1961). For the purpose of statistical analysis the PER data are based on eight rats per group.

a lowering of PER values of fish protein concentrate when treated with H_2O_2.

Amino acid supplementation of the two isolates with methionine, lysine, and threonine at the optimal levels resulted in significant improvement in their protein quality. The gain in body weight of rats fed the untreated protein isolate diet increased from 31.1 to 48.3 g and the PER from 1.43 to 2.10. In the case of H_2O_2-treated isolate supplemented with amino acids at similar levels, the gain in body weight was quite appreciable, being 32.2 g compared to 6.6 g in the unsupplemented group; the corresponding PER values were 1.38 and 0.49, respectively.

Studies on the effect of feeding groundnut cake or the groundnut protein isolates at a higher protein level of 20% in the diets have shown that the growth performance of animals could be greatly enhanced since at this higher protein level the amino acid requirements of rats for optimal growth could be met. Miller et al. (1978) reported that groundnut meal fed at 20% protein level provided nearly 90% of the essential amino acid requirements of growing rats and promoted good growth. In the present study, animals fed the groundnut cake diet showed a gain in weight of 72.2 g, this being the highest among all the groups. The untreated protein isolate group yielded a gain in weight of 62.3 g, and the isolate treated with H_2O_2 group showed a gain in weight of only 30.3 g. Thus, groups fed the cake and the untreated protein isolate at 20% protein level in the diet showed equal or even better growth performance compared to groups fed casein or SMP diets having 10% protein level. On the other hand, the H_2O_2-treated protein isolate was not nutritionally adequate even when fed at the higher level of 20% in the diet. It may be of interest that the growth response of animals fed the H_2O_2-treated isolate with amino acids (32.2 g) is comparable with that of either the protein isolate (untreated) fed at 10% (31.1 g) or the H_2O_2-treated protein isolate fed at 20% level (30.3 g). Even though the higher level of feeding protein (20%) in the diet promoted good growth, the PER values were slightly lower compared to those obtained at 10% protein level. The biological utilization of proteins is generally lower at higher levels of protein intake (Allison, 1955, 1963).

From the results of this study it could be concluded that the H_2O_2 treatment for aflatoxin removal from groundnut protein has adverse effects on the nutritional quality of the protein. This could be partly overcome by amino acid supplementation of the protein.

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Registry No. H_2O_2, 7722-84-1; lysine, 56-87-1; methionine, 63-68-3; threonine, 72-19-5; aflatoxin B_1, 1162-65-8.