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Effect of aflatoxin B_1 detoxification on the physicochemical properties and quality of ground nut meal

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Abstract

Aflatoxin B_1 (AFB_1) in groundnut meal (GNM) was detoxified up to 97% by a combination of enzymatic and physical processes. Finely-powdered defatted groundnut meal containing 1.2 mM AFB_1 per 100 g of meal was first detoxified up to 53% with 10 IU of horseradish peroxidase enzyme in the presence of hydrogen peroxide and then the meal containing 12–15% (wb) moisture was treated with microwave radiation at 1 kwt for 15 min to achieve a final 97% detoxification. A comparison was made of the treated and untreated groundnut meal quality with respect to their nitrogen solubility, total nitrogen and protein nitrogen contents, and protein composition. Rat feeding experiments were performed to study the effect of detoxification processes on ground nut meal quality as indicated by the mortality, food efficiency ratio and food conversion rate. Mean weight gains of the rats receiving the treated meals were essentially comparable to those for animals receiving aflatoxin-free diets. Overall, nitrogen-solubility of the enzyme-treated meal increases in the pH 2–6 range. Polyacrylamide gel electrophoretic patterns of the protein did not show any notable changes. Amount of protein nitrogen in the meal increased after the enzymatic treatment. Mortality rates of the young animals were high when they were fed untreated meal containing aflatoxin. Resistance increased with age, which was evidenced by the relatively lower mortality rate of the rats after prolonged feeding of aflatoxin-infected meal.

1. Introduction

Aflatoxins are a group of closely-related mycotoxins that are widely distributed in nature. The most important of the group is aflatoxin B_1 (AFB_1), which has a range of biological activities including acute toxicity, teratogenicity, mutagenicity and carcinogenicity. Detoxification of aflatoxin-contaminated foods has been a continuing challenge for the food industry. The specific detoxification method for a particular aflatoxin-infected commodity depends on several limiting factors, including moisture content, form of food and interaction of toxin with food components. Treatment conditions during detoxification should not cause any undesirable alterations to the nutritional and organoleptic qualities of the food. Earlier work (Das & Mishra, 2000) has indicated that horseradish peroxidase (HRP) can successfully detoxify authentic aflatoxin B_1 in a liquid culture up to 60% and AFB_1 spiked in defatted groundnut meal up to 53%. Staron, Thirouin, Perrin and Frere (1980) reported the use of microwave (MW) radiation in detoxification of aflatoxin. Detoxification of AFB_1 in vitro, using enzymes alone in a commodity such as GNM, may be problematic, as increase in the moisture content of the meal, which is almost an absolute requirement for enzyme activity, may cause more fungal attack. Similarly, achieving complete detoxification using microwave treatment only, may necessitate the use of harsher conditions, thereby causing qualitative deterioration of the meal. The combined use of enzymatic and microwave treatment helps better to detoxify AFB_1 than the use of any single procedure. The infected GNM was treated first with the enzyme HRP and subsequently with microwave. However, before the combined process is recommended for commercial application it is necessary that the nutritional value of such treated meal be assessed properly. Effects of aflatoxin on experimental animals provide sufficient evidence of the hazardous nature of the toxin (Palmgren & Hayes, 1987). To draw a positive correlation between the effects on animals, upon consumption of detoxified

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GNM, and the feasibility of the detoxification process is the main objective of this work.

Clifford and Rees (1967) proposed that the biochemical changes underlying the development of liver necrosis in the rat after the administration of AFB_1, within a given species of the same age, are a reflection of the ability of the toxin to interact with DNA. In the present investigation the manifestation of those biochemical changes on the overall morphology of the male Wistar rats of 2 weeks old is studied.

Contamination of oilseeds with *Aspergillus flavus* results in meals that are toxic and have altered amino acid profiles (Cherry, Dechary & Ory, 1973; Cherry, Young & Beuchat, 1975; Goldblatt, 1971). The effect of the detoxification process on GNM quality was evaluated with respect to the total nitrogen and protein nitrogen contents, protein profile on polyacrylamide gel electrophoresis, food efficiency ratio and the physiological behaviour of animals through rat feeding trials.

2. Materials and methods

Good quality groundnut seeds (*Arachis hypogaea* Cv JL- 24) were procured from a local market, powdered and defatted in Soxhlet continuous extraction apparatus to be used as the control. Male Wistar albino rats bred from the same colony were used for the rat feeding experiments. *Aspergillus flavus* ATCC 15517 was used for AFB_1 extraction. HRP enzyme and AFB_1 standards were procured from Sigma Chemicals (USA). All other chemicals were of reagent grade. A household microwave oven was used for the treatment of (GNM).

2.1. Detoxification of GNM

Finely powdered (0.25 mm particle size) defatted of ground nut seeds (100 g), containing 7.9% wb, moisture (Association of Official and Analytical Chemists [AOAC], 1995) were spiked with 1mM of AFB_1 in chloroform solution. After a homogeneous mixing, the solvent was driven off in an inert atmosphere and dried at room temperature (18–20°C) for 2–3 h. The meal was detoxified up to 53% with 10 IU of enzyme HRP in the presence of hydrogen peroxide (Das & Mishra, in press) and then subsequently by microwave treatment at 1 kwt output energy for 15 min, achieving a final 97% detoxification of the meal.

2.2. Protein quality assessment studies

Functional properties of the treated groundnut meal, with respect to the untreated GNM and untreated GNM spiked with aflatoxin B_1, were analysed using standard laboratory procedures.

To determine the nitrogen solubility profile, groundnut proteins were extracted from flours in 1 M NaCl, 20 mM sodium phosphate buffer at a ratio 1:18 (w:v, flour: extraction medium) as described by Basha and Cherry (1976). The pH of the extract was adjusted to 2, 4, 6, 8, 10 using appropriate amounts of 1 N–6 N HCl and 1 N–6 N NaOH. After 1 h of constant stirring on a magnetic stirrer at 25°C the slurry was centrifuged at 15,000 *g* for 30 min at 4°C and filtered through Whatman No.1 filter paper to obtain a clear extract. A 25 ml samples of each extract was analysed for nitrogen content by the Kjeldahl method (AOAC, 1995). The percent soluble nitrogen in each sample was calculated and plotted against the corresponding pH values.

The powdered sample was also analysed for total nitrogen content by the Kjeldahl method. To know the amount of non-protein nitrogen in GNM, a known quantity of powdered material was dissolved in ice cold 10% trichloroacetic acid. Proteins were precipitated and non-protein nitrogen was extracted. After centrifugation, the precipitate was washed and analysed for nitrogen content. Multiplying the nitrogen value by 5.7 (conversion factor for ground nut protein) gave the true protein content. Deduction of protein nitrogen gave from total nitrogen gives the non-protein nitrogen content of the sample (Pelett and Young, 1980).

Electrophoretic mobility of the peanut proteins was determined on 10% poly acrylamide gels (PAG). The samples, containing 200–500 μg of protein (pH 7.7), were electrophoresed according to the method outlined by Cherry et al. (1973). From each of the sample proteins, 50 μl were taken to load into the wells of the gel containing sodium dodecyl sulfate (SDS) as anionic detergent. The protein-SDS complex carries net negative charges and hence moves towards the anode. The separation is based on the size of the protein.

2.3. Rat feeding studies

This experiment was carried out to study the relative toxicity of treated and untreated GNM supplemented with control diet. The control diet consisted of carbohydrate 40%, ground nut oil 7–8%, non-nutritive cellulose 2%, salts 4%, vitamins 1% and GNM 45%. Individual cages were provided with feeders. To each group of 8 rats, 100 g diet was provided per day. Diet A consisted of GNM containing 1.2 mM AFB_1. Diet B consisted of the control diet and the GNM with AFB_1 detoxified by HRP only and Diet C consisted of GNM detoxified by both HRP and microwave treatment. For the rat feeding test the animals of almost equal body weight were housed in four individual groups and fed four types of meal. Food consumption and body weights were recorded daily. Mortality of the animals was noted. The surviving animals were sacrificed after certain intervals and organs were weighed. Food efficiency ratio was calculated as gain in body weight per gram of the food consumed by the experimental rats. Statistical analysis of

Table 1
Moisture, protein and nitrogen contents (%) of GNM before and after decontamination treatment[b]

Meal	Treatment	Moisture (%) w.b.		Protein (%)[a]		Aflatoxin content (μg)	
		Before	After	Before	After	Before	After
Control	GNM with no treatment	7.9	7.9	44.7	44.7	0	0
A	GNM + AFB_1 with no treatment	10	10	44.7	44.6	190	190
B	GNM + AFB1 + HRP	15	15	44.7	45.2	190	89.30
C	GNM + AFB_1 + HRP + MW	15	8.1	44.7	44.5	89.30	5.70

[a] Nitrogen to protein conversion factor for GNM was 5.7.
[b] Nitrogen content was unaltered before and after the treatment i.e. 7.89.

the results was done and reproducibility was checked by performing the experiments in triplicate.

3. Results and discussion

Table 1 presents the compositions of different diets used in this investigation. The detoxification methods described substantially reduced the aflatoxin from the contaminated GNM as measured qualitatively by TLC assay and quantitatively by the spectroflurometric method (Heathcote & Hibbert, 1978). The GNM contained 7.9% protein nitrogen which remained unaffected by the detoxification treatments. The enzyme treatment of the meal followed by microwave treatment was effective with almost complete (97%) removal of AFB_1 present without deteriorating the organoleptic qualities of the meal. The protein composition and mobility, as evidenced by the band patterns of PAGE of the meals A and B with respect to control, did not show much change when the gel was stained with amido black, except for the appearance of some faint bands in the case of meal B i.e. infected GNM treated by HRP only. However, microwave treatment, even under the mildest conditions, causes change in the protein as indicated by differences in the electrophoretic mobility of the protein subunits in the case of meal C.

Nitrogen solubility curves (Fig. 1) for contaminated meals and treated meals also show satisfactory results. Nitrogen solubility increases after HRP treatment of the aflatoxin B_1 contaminated GNM in the pH range 4–6. However, at the acid and base extremes there were no indications of any changes in the solubility profiles of the meal samples with respect to control set. The typical minimum solubility at pH 5, normally associated with ground nut proteins (Conkerton, Chapital, Lee & Ory, 1980), was also unaltered. At this point, GNM was insoluble in all the treatment conditions.

A comparative evaluation of the capacity of AFB_1-contaminated GNM and the same after detoxification to initiate changes in the mean body weight, and liver to body weight ratio was made by 2 week-old rat feeding tests (Table 2). The mean body weight of the rats was increased when fed with all the 4 types of diet up to the 6 th week. But, after 6 weeks, the mean body weight starts decreasing in the case of rats fed with diet A, i.e. containing 1.2 mM AFB_1 without any treatment. The mean body weight remained unaltered in the case of rats fed with Diet B, that is the diet supplemented by HRP-treated AFB_1 and increased for the two other sets. The liver to body weight ratio was measured at 2 week intervals up to 8 weeks. There was a marked increase in the liver to body weight ratio in the case of rats fed with AFB_1-infected meal for 8 weeks continuously. Data on mortality, body weight, food consumption and food efficiency ratio are shown in the Table 3. Food consumption, as well as weight gain and the corresponding food efficiency ratio of individual rats indicated, that the Diet C was highly comparable to the control diet and had no lethal or deleterious effect on the animal. One rat out of 8 died after prolonged feeding for 4 months with Diet B indicating its superiority over the Diet A containing 190 μg of AFB_1 per 100 g which caused death of 5 animals out of 8 within 7 weeks of feeding. The surviving rats suffered from weight loss, severe de-hairing,

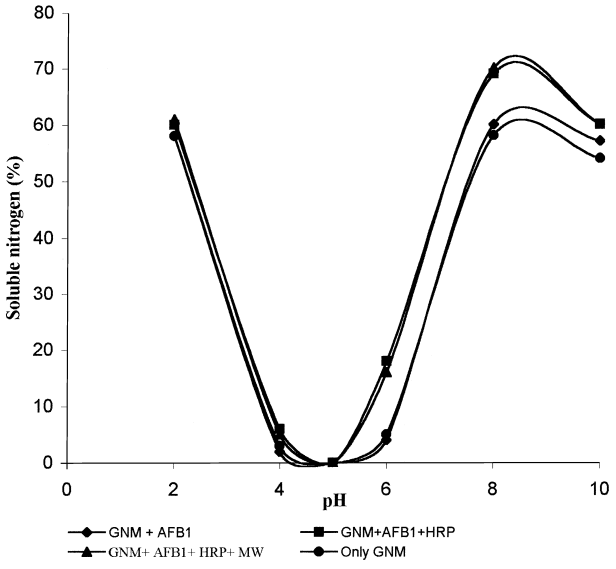

Fig. 1. Nitrogen solubility of ground nut meal and detoxified product as a function of pH.

Table 2
Effect on body weight and liver to body weight ratio in rats after feeding test for 8 weeks

Diet	Amount of AFB_1 in diet (μg/100 g)	Mean body weight (g)				Liver to body weight ratio (g/100 g body weight±S.E.)			
		Period of feeding (week)							
		2	4	6	8	2	4	6	8
Control	0	38	48	58	64	3.21±0.91	3.20±0.99	3.10±1.23	3.24±0.52
A	190	33	43	51	48	3.20±0.16	3.9±0.88	4.00±0.91	4.00±0.71
B	89.3	35	40	51	51	3.48±0.82	3.31±0.71	3.32±0.95	3.32±0.96
C	5.7	32	45	57	65	3.20±0.55	3.20±0.39	3.21±2.20	3.28±0.87

Table 3
Effect of rat feeding test on mortality, body weight, food consumption and food conversion after 4 months

Group[b]	Mortality	Initial weight (g) at 2 wks	Final weight (g)	Weight gain (g)	Food consumption (g)	Food efficiency ratio
Basal diet + GNM	Nil	38±3	300±18	262±15	2550±50	0.103±0.003
A (basal diet + GNM + AFB_1)[a]	5/8	33±3	270±21	201±18	2213±91	0.091±0.004
B (basal diet + GNM + HRP + MW)	Nil	32±3	301±13	269±10	2561±89	0.105±0.002
C (Basal diet + GNM + HRP)[a]	1/8	35±2	289±15	254±13	2520±62	0.100±0.003

[a] Data are the mean of live animals±S.E.
[b] Each group consists of 8 rats.

Table 4
Mean relative organ weights (g/100 g body weight±S.E.) of rats fed with treated and untreated meals after 4 months

Group[a]	Liver	Kidney	Heart	Spleen
Basal diet + GNM	3.270±0.081	0.628±0.018	0.309±0.006	0.202±0.008
B (basal diet + GNM + HRP)	3.297±2.1	0.627±0.015	0.308±0.051	0.192±1.01
C (basal diet + GNM + HRP + MW)	3.268±0.019	0.627±1.210	0.308±1.005	0.200±0.09
A (basal diet + GNM + AFB_1)	3.722±0.912	0.680±0.008	0.309±0.089	0.222±0.065

[a] Each group consists of 8 rats.

especially at the dorsal surface of the head, and eyes became blurry with overall lethargic dull appearance. Water uptake, too, was much less for the group fed on Diet A. The organ weights (Table 4) of liver and kidney of animals fed on the meal infected with AFB_1 were significantly higher than those of the groups fed on Diets B or C, or the control diet. Deposition of yellow-coloured adipose tissue on the kidney surface was noticeable in this group. The weights of other organs viz., heart and spleen, of the experimental animals did not show much difference from the control group. This result supports earlier findings about the action of AFB_1 on rat liver reported by several scientists (Dollear, Mann, Codifier, Gardner, Koltun & Vix, 1968; Hamilton, 1984). In those cases, AFB_1 produced hepatic carcinoma after prolonged feeding tests.

4. Conclusion

In vitro detoxification of AFB_1 in groundnut meal by a combination of HRP enzyme and microwave treatments was quite satisfactory. This treated meal was highly acceptable by the animals and had little or no deleterious effect on them when compared to toxic meals. The protein quality, as indicated by nitrogen solubility and nitrogen content of the meals, and the composition found from SDS-PAGE, remained almost unaltered at the end of the detoxification process. Microwave treatment had a dual effect on the meal. The high moisture content of the GNM is a prerequisite for the efficient enzymatic detoxification of the meal but that again favours further fungal attack. The exposure of the enzymatically-detoxified GNM to microwave

treatment decreases the moisture content of the meal (7%), thereby preventing further fungal attack and the use of fungistat. Moreover, the initial high moisture content of the enzyme-treated GNM favours more heat energy absorption during the subsequent microwave treatment. The experiment also reveals that an adequate amount of protein, needed for healthy growth and survival of the animals, was available from the detoxified GNM. Further feeding trials are, however, necessary on farm animals and humans to support the suitability of the detoxified GNM as feed and food.

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