Destruction of Aflatoxins in Peanut Protein Isolates by Sodium Hypochlorite

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ABSTRACT

Sodium hypochlorite has been tested for destruction of aflatoxins during the preparation of peanut protein isolates from raw peanuts and defatted peanut meal. The treatments were evaluated by determination of the aflatoxins in the products by thin layer chromatography. Effects of sodium hypochlorite concentration, reaction pH, temperature, and time were studied. Results show that both the sodium hypochlorite concentration and pH are important factors in reducing the concentration of aflatoxins in the protein isolates to nondetectable levels. The treatment with 0.4% sodium hypochlorite at pH 8 produced protein isolates with trace amounts of aflatoxins B_1 and B_2 from ground raw peanuts containing 725 ppb aflatoxin B_1 and 148 ppb aflatoxin B_2, whereas untreated protein isolates contained 384 ppb aflatoxin B_1 and 76 ppb aflatoxin B_2. At pH 9, 0.3% sodium hypochlorite reduced the aflatoxin B_1 content in the protein isolates from 300 ppb to below detectable quantities and the aflatoxin B_2 content from 52 ppb to 2 ppb. Similar results were obtained at pH 10 for 0.3% sodium hypochlorite concentration. In the case of defatted peanut meal which contained 136 ppb aflatoxin B_1 and 36 ppb aflatoxin B_2, 0.25% sodium hypochlorite concentration at pH 8 (0.20% at pH 9; 0.15% at pH 10) reduced both the aflatoxin B_1 and B_2 contents to below detectable quantities in protein isolates as compared to aflatoxin levels of ca. 75 ppb B_1 and 17 ppb B_2 in the untreated protein isolates. Reaction temperature and time did not affect the destruction of aflatoxins significantly.

INTRODUCTION

The aflatoxins are a group of highly toxin metabolites produced by most strains of the fungus *Aspergillus flavus Link ex Fries* and the closely related *Aspergillus parasitcus* (1). In view of the hepatotoxic and carcinogenic effects of aflatoxins upon laboratory and domestic animals (2) and their presence in certain foodstuffs, they are considered a potential threat to food safety and human health (3,4). Aflatoxins recently have been suggested as possible etiologic agents in certain human diseases, including liver cancer (5,6). Therefore, much attention has been devoted to various methods for eliminating or lowering to acceptable levels the concentration of aflatoxins which sometimes occurs in certain agricultural commodities. Primarily, these efforts have centered around removal of the aflatoxins by the physical separation of aflatoxin contaminated products from those free of contamination or by chemical destruction of aflatoxins (7).

Aflatoxins have been detected in peanuts and in the meal produced from this commodity when crushed for oil extraction (8-10). The current processing practices, mechanical expression and extraction with hexane, retain, in the defatted meal, a considerable proportion of the toxin that may be present in the seed. Since alkaline refining and bleaching of peanut oil removed essentially all of the toxin that remained with the oil during processing (11), contaminated kernels usually are crushed for oil. The remaining contaminated peanut meal may be unacceptable for domestic use in either food or feed which reduces the intrinsic economic value of the peanut crop as a whole. However, this can be exported to many foreign countries provided the aflatoxin content is indicated on the freight bill.

Because of the growing importance of peanuts as a source of low cost protein to supplement diets for alleviation of protein malnutrition, particularly in the developing countries where aflatoxin contamination of peanuts is most severe, it is important to eliminate or remove aflatoxins from contaminated peanuts. Destruction by chemical means shows the greatest potential for reducing aflatoxin content. Several chemical agents have been suggested as effective in destroying aflatoxins (12,13). Some of these reagents have been used experimentally for destruction of aflatoxins in peanut meal (14-16) but have not been demonstrated to be commercially feasible, with the exception of ammoniation which is being used to salvage aflatoxin contaminated cottonseed and cottonseed meal (17).

To date, the most common and effective reagent reported in the literature is sodium hypochlorite (NaOCl). This inexpensive chemical has been recommended by Trager and Stoloff (12) and Stoloff and Trager (18) as a safety measure for disposal of contaminated materials in laboratories engaged in aflatoxin research. Yang (19) also confirmed the destruction of aflatoxins by sodium hypo-

TABLE I

Effect of NaOCl Concentration and pH upon Destruction of Aflatoxins in Protein Isolates Prepared from Raw Peanuts[a]

Concentration (%)	Residual aflatoxins in protein isolates (ppb)					
	B_1	B_2	B_1	B_2	B_1	B_2
	(pH 8)		(pH 9)		(pH 10)	
0	384	76	300	60	300	60
0.05	220	62	133	32	68	17
0.10	120	35	50	13	25	5
0.15	30	8	20	3	15	3
0.20	20	5	10	3	7	3
0.25	14	4	trace	3	trace	3
0.30	8	3	0	0	0	0
0.35	0	3	0	0	0	0
0.40	0	0	0	0	0	0

[a]Reaction temperature 60 C. Reaction time 30 min.

TABLE II

Effect of Temperature and pH upon Destruction of Aflatoxins in Protein Isolates Prepared from Raw Peanuts by Sodium Hypochlorite[a]

Temperature (± 2 C)	Residual aflatoxins in protein isolates (ppb)					
	B_1	B_2	B_1	B_2	B_1	B_2
	(pH 8)		(pH 9)		(pH 10)	
25	125	32	27	66	14	4
35	121	36	30	8	16	5
45	114	30	30	12	12	4
55	128	26	23	6	22	9
65	123	29	24	9	37	15
75	118	26	32	9	62	12

[a]NaOCl concentration 0.1%. Reaction time 30 min.

TABLE III

Effect of Time and pH upon Destruction of Aflatoxins in Protein Isolates Prepared from Raw Peanuts by Sodium Hypochlorite[a]

Time (min)	Residual aflatoxins in protein isolates (ppb)					
	B_1	B_2	B_1	B_2	B_1	B_2
	(pH 8)		(pH 9)		(pH 10)	
15	132	26	27	6	37	15
30	126	30	23	6	52	10
45	122	26	24	9	44	12
60	128	26	29	7	38	16

[a]NaOCl concentration 0.1%. Reaction temperature 60 C.

chlorite and commercial bleaches containing NaOCl.

The purpose of the present study was to determine the efficacy of sodium hypochlorite in destroying aflatoxins in contaminated raw peanuts and peanut meal undergoing processing for the preparation of protein isolates. The effects of a number of parameters, such as NaOCl concentration, reaction pH, temperature, and time, upon the reaction of NaOCl with aflatoxins were investigated.

EXPERIMENTAL PROCEDURES

Materials and Methods

Contaminated peanut splits of the Spanish variety were used in the experiments. Split peanuts were blanched mechanically without the use of heat to remove most of the skins and germs. The blanched kernels then were ground with an Urschel mill (Comitrol 3600) equipped with a medium head. The ground raw peanuts contained 725 ppb aflatoxin B_1 and 148 ppb aflatoxin B_2. No aflatoxins G_1 and G_2 were detected. Other analytical data on the ground raw peanuts include moisture, 7.1%; nitrogen, 5.2%; oil 43.2%; ash, 2.85%; and crude fiber, 2.4%.

The defatted peanut meal was a solvent-extracted sample chosen because it contained 136 ppb aflatoxin B_1 and 36 ppb aflatoxin B_2 and no aflatoxin G_1 and G_2. Meal moisture was 10.0%; nitrogen, 9.8%; oil, 0.3%; ash, 4.1%; crude fiber, 3.3%; and nitrogen solubility, 99.5%.

Peanut protein isolates were prepared from the above described ground raw peanuts according to the aqueous extraction process developed by Rhee, et al (20). Protein isolates from defatted peanut meal were prepared as described in an earlier report (21). Raw peanuts, meal, and protein isolates were assayed for aflatoxin by the method of Pons, et al. (22). Moisture content of the isolates was taken into consideration to make up 85% acetone for aflatoxin extraction. Unless otherwise specified, the data are the average of three replicate analyses.

NaOCl Treatment

In the case of raw peanuts, a calculated amount of 5% sodium hypochlorite solution was added to the aqueous suspension at 60 ± 2 C after the pH was adjusted to the desired values. Extraction was continued for 30 min. The pH was maintained at the desired values by adjustments at 10 min intervals during extraction. In the case of peanut meal, extraction was carried out at room temperature after the addition of the NaOCl solution. protein was precipitated at the isoelectric pH after centrifugation of the alkaline extract. Protein isolates also were prepared without NaOCl treatment. The residual aflatoxin in the protein isolates, treated and untreated, was determined. The effect of reaction temperature and time at three different extraction pHs upon the destruction of aflatoxins was studied with a NaOCl concentration of 0.1%, and the results reported are the average of three replicate analyses.

RESULTS AND DISCUSSION

Studies with Raw Peanuts

Effect of NaOCl concentration: The effect of sodium hypochlorite concentration upon the total aflatoxins at the 3 extration pHs of 8, 9, and 10 is shown in Table I. Protein isolates prepared without hypochlorite treatment at pH 8 contained 384 ppb of B_1 and 76 ppb of B_2, whereas the isolates prepared at pHs 9 and 10 contained 300 ppb of B_1 and 60 ppb of B_2. As reported earlier (21), this represents ca. 55% of the total toxins present in the starting materials. At pH 8, both aflatoxins B_1 and B_2 were destroyed completely at a concentration of 0.40% NaOCl. Treatment with 0.35% NaOCl reduced the B_1 content to trace amounts and B_2 content to 3 ppb. At pHs 9 and 10, no B_1 and only 2 ppb B_2 were detected in the protein isolates at a concentration of 0.30% NaOCl. At 0.25% NaOCl concentration level, B_1 was detected but could not be quantitated at either pH.

The results presented in the Table I also indicate that pH has an effect upon the destruction of aflatoxin but is more marked at lower NaOCl concentrations. This point is illustrated by the effects of 0.05% and 0.1% NaOCl at pHs 8, 9, and 10. At pH 8, for a 0.05% NaOCl concentration, B_1 was reduced to 220 ppb and B_2 to 62 ppb, a reduction of about 37% of the total toxins in the untreated protein isolates. At

TABLE IV

Effect of NaOCl Concentration and pH upon Destruction of Aflatoxins in Protein Isolates Prepared from Defatted Peanut Meal[a]

Concentration (%)	Residual aflatoxins in protein isolates (ppb)					
	B_1	B_2	B_1	B_2	B_1	B_2
	(pH 8)		(pH 9)		(pH 10)	
0	73	21	79	15	79	15
0.05	41	16	37	11	8	3
0.10	14	4	7	2	6	trace
0.15	7	2	5	trace	0	0
0.20	3	trace	0	0	0	0
0.25	0	0	0	0	0	0

[a]Reaction temperature 24 C. Reaction time 30 min.

TABLE V

Effect of Temperature and pH upon Destruction of Aflatoxins in Protein Isolates Prepared from Defatted Peanut Meal by Sodium Hypochlorite[a]

Temperature (± 2 C)	Residual aflatoxins in protein isolates (ppb)					
	B_1	B_2	B_1	B_2	B_1	B_2
	(pH 8)		(pH 9)		(pH 10)	
25	14	4	7	4	5	2
35	10	2	6	4	6	3
45	10	6	8	3	6	2
55	16	2	7	4	7	3
65	14	4	8	4	6	4
75	10	4	8	5	8	3

[a]NaOCl concentration 0.1%. Reaction time 30 min.

pH 9, for the same concentration of NaOCl, B_1 was reduced to 133 ppb and B_2 to 32 ppb, which corresponds to a reduction of ca. 54% of the total toxin in the untreated protein isolates. At pH 10, the corresponding reduction in B_1 and B_2 was 68 ppb and 17 ppb, respectively. This represents a reduction of ca. 76% of the total toxins in the untreated protein isolates. At 0.1% NaOCl concentration, there was more destruction of aflatoxins at pHs 9 and 10 than at pH 8.

Effect of reaction temperature and time: From the data summarized in Table II, it is evident that, at a NaOCl concentration of 0.1%, temperature did not have any significant effect upon the destruction of aflatoxins in the protein isolates prepared at pHs 8 and 9. However, at pH 10, a significantly higher aflatoxin content was observed at temperatures above 55 C than at lower temperatures. Since the mode and rate of decomposition of NaOCl can be influenced by temperature, concentration, pH, and catalysts (23), it seems possible that, at higher pHs, higher temperatures may favor a reaction other than the one responsible for the oxidizing action of hypochlorite.

In studying the effect of time upon the destruction of aflatoxins by NaOCl, a period of 5-6 min usually was required to bring the pH to the desired value. Extraction was carried out for a desired time interval with necessary occasional pH adjustments. The effect of time upon the destruction of aflatoxins by NaOCl is given in Table III which indicates that maximum destruction has been achieved in 15 min and additional contact time serves no purpose. It is possible that less than 15 min contact time may be adequate for destruction of aflatoxins by NaOCl.

Studies with Peanut Meal

Effect of NaOCl concentration: Table IV shows the effect of NaOCl concentration upon aflatoxins in defatted peanut meal. Treatment of peanut meal with 0.25, 0.20, and 0.15% NaOCl concentrations at the extraction pHs 8, 9, and 10, respectively, reduced both aflatoxins B_1 and B_2 to undetectable amounts from aflatoxin contents of about 75 ppb B_1 and 17 ppb B_2 in the untreated isolates. Again, as in the case of raw peanuts, pH seems to influence the destruction of aflatoxins by NaOCl more markedly at lower concentration levels. For example, treatment with 0.1% NaOCl at pH 8 reduced B_1 and B_2 contents to 14 and 4 ppb, respectively. Untreated protein isolates prepared at this pH contained 73 ppb of B_1 and 21 ppb of B_2. At pH 9, for the same concentration of NaOCl, B_1 and B_2 contents were 7 ppb and 2 ppb, respectively. Protein isolates prepared without NaOCl treatment contained 79 ppb of B_1 and 15 ppb of B_2. At pH 10, 6 ppb of B_1 and 2 ppb of B_2 were detected at 0.1% NaOCl concentration level. The influence of pH on the destruction of aflatoxin by NaOCl is even more striking at 0.05% concentration level than at 0.1% level. At this concentration, at pH 10, ca. 90% reduction in aflatoxin content was obtained, whereas at pHs 8 and 9, aflatoxin reduction was less than 50%.

Effect of reaction temperature and time: The effect of reaction temperature and time upon destruction of aflatoxins by NaOCl at the three extraction pHs is shown in Tables V and VI which indicate that these parameters within the range studied did not produce a significant effect upon the destruction of aflatoxins by NaOCl. As in the case of raw peanuts, maximum destruction was achieved in 15 min. The higher aflatoxin content at higher temperatures at pH 10, observed in raw peanuts (Table II), was not observed in the case of defatted meal under similar conditions. This may be due to the lower content of aflatoxins in the meal.

Residues, the by-products in the protein isolate preparation, contained no aflatoxins at higher NaOCl concentrations. Since the residues were rich in carbohydrates and minerals, NaOCl treated residues could be used in animal feeds.

The possiblity of NaOCl acting either as an oxidizing agent or as a chlorinating agent suggested testing the treated protein isolates for possible formation of N-chloro-compounds which may be toxic. A subsequent literature survey indicated that none of the earlier investigations

TABLE VI

Effect of Time and pH upon Destruction of Aflatoxins in Protein Isolates Prepared from Defatted Peanut Meal by Sodium Hypochlorite[a]

Time (min)	Residual aflatoxins in protein isolates (ppb)					
	B_1	B_2	B_1	B_2	B_1	B_2
	(pH 8)		(pH 9)		(pH 10)	
15	15	5	7	3	6	2
30	15	3	8	3	5	3
45	15	6	7	5	6	4
60	17	5	6	3	7	2

[a]NaOCl concentration 0.1%. Reaction temperature 24 C.

(24,25) characterized the products from the action of NaOCl on proteins as having N-chloro compounds. However, the investigations did indicate that, in acid solutions, chlorination predominates over oxidation, and, in alkaline solutions, oxidation is more marked. The absence of N-chloro-compounds in the protein isolates as tested by the starch-iodide reaction of Rydon and Smith (26) tends to corroborate the earlier reports.

The possible chemical changes that follow NaOCl treatment of aflatoxins under the conditions of the present study are not known with certainty at the present time. In all treatments in which aflatoxins were not detected, the TLC plates after development in chloroform-acetone (9+1) were sprayed with 2,4-dinitrophenylhydrazine proposed by Crisan (27) as a confirmatory test for the cyclopentenone carbonyl group of aflatoxin B_1. This reagent gives an orange to yellow color with aflatoxin B_1. The absence of yellow-orange color on the TLC plates after spraying with 2,4-dinitrophenylhydrazine indicated that the 5-membered cyclopentenone ring from the original aflatoxin B_1 was not retained after treatment with NaOCl. Trager and Stoloff (12) suggested that the reactions appear to be primarily addition and oxidation involving the olefinic double bond of the terminal furan ring and oxidation involving the substituted O-coumaric acid formed on opening of the lactone ring. The UV absorption curves of aflatoxins extracted from hypochlorite treated isolates showed a complete loss of major peak at 363 nm (28). Similar changes in the UV absorption after treatment of aflatoxins with NaOCl and commercial bleaches also were reported (19). The changes in the UV absorption spectra and the negative test for the cyclopentenone carbonyl group suggest that more drastic changes ensued, resulting in extensive breakdown of the molecules. Since all treatments are carried out under alkaline conditions, it may be postulated that, in alkaline solutions, the lactone ring common to aflatoxins B_1 and B_2 hydrolyzes to the hydroxy acid form which may be susceptible to oxidation by NaOCl.

It may be concluded from the results described in this report that it would be possible to destroy aflatoxin to nondetectable levels or to reduce the aflatoxin level in the protein isolates prepared from either raw peanut or defatted peanut meal to within the acceptable levels.

Investigation in prospect: Although the earlier reports have shown that the reaction products of aflatoxins after treatment with NaOCl were nontoxic to chick embryo and tissue culture (12) and to ducklings (19), it is not known at this point whether the NaOCl treatment of raw peanuts or peanut meal introduced any new toxic compounds in the protein isolates. Animal feeding experiments are needed to provide biological data on toxicity and nutritional potential of the hypochlorite treated protein isolates described in this work.

Preliminary studies show that the quality of treated protein isolates is not degraded when these isolates are analyzed chemically for nitrogen solubility and available lysine. Results will be reported in a subsequent publication from this laboratory.

Experiments are in progress in our pilot plant facilities to determine the commercial feasibility of the findings reported in this communication.

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