Processing Edible Peanut Protein Concentrates and Isolates to Inactivate Aflatoxins

K.C. RHEE, K.R. NATARAJAN,[1] C.M. CATER, and K.F. MATTIL, Food Protein Research and Development Center, Texas A&M University, College Station, Texas 77843

ABSTRACT AND SUMMARY

Experiments were conducted to study the efficacy of some oxidizing or other reactive chemicals for destruction of aflatoxins in conjunction with the aqueous extraction process for the production of peanut protein concentrates and/or isolates directly from contaminated raw peanuts. The chemicals tested included acetone, isopropyl alcohol, methylamine, hydrogen peroxide, benzoyl peroxide, ammonia gas, and sodium hypochlorite. Among these chemicals, hydrogen peroxide, benzoyl peroxide, and sodium hypochlorite showed very effective destruction of aflatoxins during the aqueous extraction process of infected peanuts. However, the use of benzoyl peroxide may pose some difficulties because it is not readily soluble in the aqueous suspensions. It was therefore concluded that aflatoxins can be effectively destroyed during the aqueous processing of peanuts by properly utilizing either sodium hypochlorite or hydrogen peroxide to produce either peanut protein concentrates or isolates.

INTRODUCTION

Traditionally, peanuts have been grown throughout the world as an oilseed crop for export or processing for production of edible oil with the protein residue in the form of oil cake being used for animal feed. The United States is the only major peanut producing country where the majority of peanuts produced are consumed as food in the form of roasted peanuts, peanut butter, candies, and snacks. However, there is considerable world-wide interest in the utilization of peanuts in ways other than such conventional uses mentioned. This great interest has resulted from the grave concern over the lack of adequate protein to provide needed nourishment for large segments of the world's population in the years ahead.

One of the newest and most promising directions for peanut utilization is through the production of peanut protein concentrates and/or isolates which can be used in a variety of food formulations. There are two basic approaches for producing peanut protein concentrates and isolates: the first method is the extraction of proteins from defatted peanut meal after the oil is removed from peanuts using an expeller followed by solvent extraction; the second is the simultaneous separation of peanut protein and oil directly from raw peanuts using an aqueous medium (1). The latter method has certain advantages over the former. One of the most important is the fact that various solvents and oxidizing and other reactive chemicals can be incorporated into the processing systems to extract or inactivate aflatoxins which may occur in some peanuts (2,3). As reported by Parker and Melnick (4) conventional processing practices, either by mechanical means or by extraction with hexane, leave, in the defatted meal, the vast majority of any aflatoxin that may be present in the raw peanuts.

Several chemicals such as acetone, isopropyl alcohol, hydrogen peroxide, methylamine, benzoyl peroxide, ammonia, and sodium hypochlorite have been suggested as being effective in extracting or destroying aflatoxins (5,6). Some of these chemicals have been used experimentally for destruction of aflatoxins in peanut meal (7,8) but have not been demonstrated to be commercially feasible, with the exception of ammoniation which is being used to salvage aflatoxin contaminated cottonseed meal (9). Todate, the most common and effective chemical reported in the literature is sodium hypochlorite. This has been recommended by Trager and Stoloff (5) and Stoloff and Trager (10) as a safety measure for disposal of contaminated materials in laboratories engaged in aflatoxin research. The effectiveness of sodium hypochlorite and commercial bleaches that contain sodium hypochlorite in destroying aflatoxins was later confirmed by Yang (11).

There are a number of publications which have discussed the effectiveness of various chemicals in extracting or destroying aflatoxins in peanut and other agricultural products (12). The objectives of this study therefore were to determine the efficacy of some of these chemicals in removing or destroying aflatoxins in contaminated peanuts in the aqueous extraction process for the preparation of peanut protein concentrates and/or isolates.

EXPERIMENTAL PROCEDURES

Materials and Methods

Aflatoxin-contaminated Spanish peanuts were hand picked and used in the experiments. Peanuts were mechanically blanched without the use of heat to remove most of the skins and germs. The blanched kernels were then ground with an Urschel Mill (Comitrol 3600) equipped with a medium head (opening size 0.03 in.). The ground sample contained 8.2% moisture, 43.6% oil (as-is basis), 5.2% nitrogen (as-is basis), 2.9% ash and 2.5% crude fiber. Aflatoxin content of this material is presented in Table I.

Peanut protein concentrates and oil or peanut protein isolates and oil were produced from the ground aflatoxin-contaminated peanuts according to the basic procedures described by Rhee et al. (1,13). Briefly, the concentrate process involved extraction of ground peanuts (25 g) with

TABLE I

Distribution of Aflatoxins Among Various Fractions Obtained from Aqueous Extraction Process

Fraction	Aflatoxin content, μg/kg[a]		
	B_1	B_2	Total
Starting material	820	215	1035
Concentrate process			
Concentrate	713(87)[b]	167(78)[b]	880(85)[b]
Oil	50 (6)	22(10)	72 (7)
Whey	57 (7)	26(12)	83 (8)
Isolate process			
Isolate	451(55)	108(50)	559(54)
Fibrous residue	196(24)	52(24)	248(24)
Oil	50 (6)	33(15)	83 (8)
Whey	123(15)	22(10)	145(14)

[a]Calculated from weight of material recovered.

[b]The numbers in parenthesis indicate the percentage of aflatoxin present in starting material which was found in the fraction.

[1]Present address: Assistant Professor of Chemistry, Presidency College, Madras 600 005, India

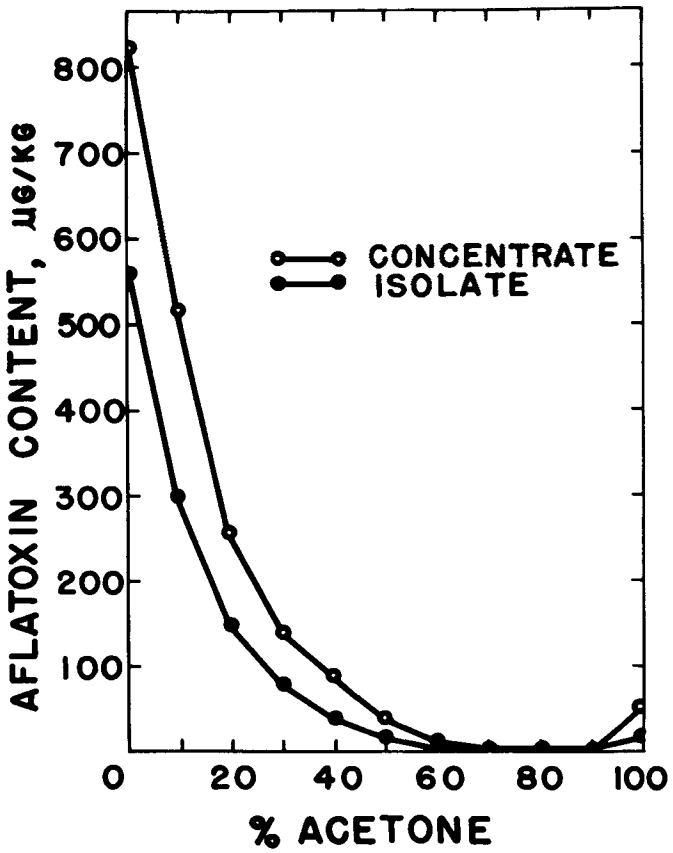

FIG. 1. Effect of acetone concentration in process water on the aflatoxin content of peanut protein concentrate and isolate.

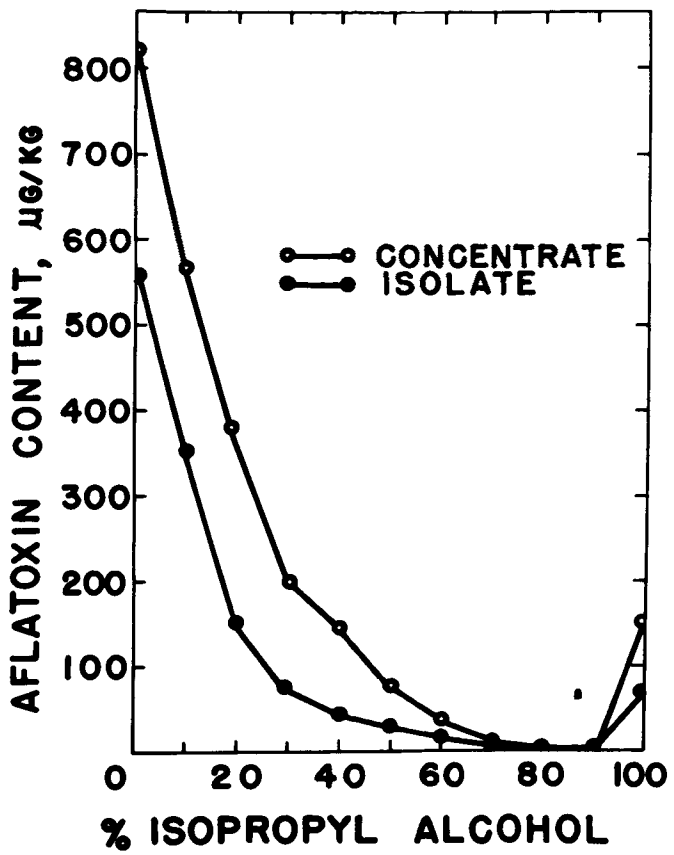

FIG. 2. Effect of isopropyl alcohol concentration in process water on the aflatoxin content of peanut protein concentrate and isolate.

six parts water (containing the various chemicals to be tested) at pH 4 for 30 min at 55-60 C followed by centrifugation at 4000 rpm for 15 min to separate protein concentrate, oil, and whey fractions. The isolate process involved extraction of ground peanuts (25 g) with six parts of water (containing test chemicals) at pH 8 for 30 min at 55-60 C followed by centrifugation at 4000 rpm for 15 min to separate the insoluble fibrous residue from the liquid extract. The pH of the liquid extract was then adjusted to 4 to precipitate the protein followed by a second centrifugation to separate protein isolate, oil, and whey fractions. Aflatoxin contents of raw material and various fractions obtained from the aqueous extraction processes were determined chromatographically by the method of Pons et al. (14). Briefly, the procedure involved extraction of the aflatoxins from the samples with 85% (v/v) aqueous acetone, initial purification by precipitation with lead acetate, partitioning of aflatoxins into chloroform, final purification on an acidic alumina column, separation of aflatoxins on this layer chromatographic plates coated with Adsorbosil-1, and visual evaluation of the intensity of fluorescence of thin spots viewed under long-wave ultra violet light. Unless otherwise specified, the data presented represent the average of three replicate analyses.

RESULTS AND DISCUSSION

Distribution of Aflatoxins

When aflatoxin-contaminated ground peanuts were subjected to an aqueous extraction process without the use of any chemicals, except sodium hydroxide and/or hydrochloric acid for the purpose of pH adjustment, aflatoxins were distributed among various fractions as summarized in Table I. In the case of the concentrate process, ca. 85% of the total aflatoxin remained in the concentrates. Crude oil contained ca. 7% and whey ca. 8% of the initial toxin present in the starting material. In the case of isolate process, the average aflatoxin distribution was as follows: isolate, 54%; fibrous residue, 24%; crude oil, 8%; and whey, 14%.

It is clear from the data that the majority of the toxins remained in the solid fractions while relatively small amounts were extracted into liquid fractions. In earlier reports, Smith (15), Van der Berg (16), and Basappa et al. (17) also made comparable observations that the majority of the toxins remained with the protein fractions. These results clearly indicate that the aqueous extraction process alone cannot be effectively used as a means of removing aflatoxins from contaminated peanuts; instead, the aqueous media should be considered as a carrier of necessary chemicals which remove or inactivate aflatoxins during processing. The results of the use of several chemicals to extract or destroy aflatoxin from ground raw peanuts in the aqueous extraction process were the following.

Effect of Aqueous Acetone

As illustrated in Figure 1, acetone is a very effective solvent in reducing aflatoxin content of either protein concentrates or isolates. The most effective concentration ranged from ca. 65% to 90% acetone (w/w) in process water. The effectiveness of acetone in removing aflatoxins gradually decreased as the acetone concentration decreased. Also, 100% acetone was less effective than 65-90% acetone mixtures with water.

It has been reported previously that aqueous acetone containing 10% water by weight is an effective solvent for reducing aflatoxin content of defatted cottonseed and peanut meals (18). Also the use of acetone containing 25-30% water has been reported to remove aflatoxins from mold-damaged, flaked cottonseed meats (19).

The use of acetone in the aqueous extraction process offers some advantages and some disadvantages. Two of the obvious advantages are: (a) virtually complete removal of aflatoxins under suitable conditions, and (b) less likelihood

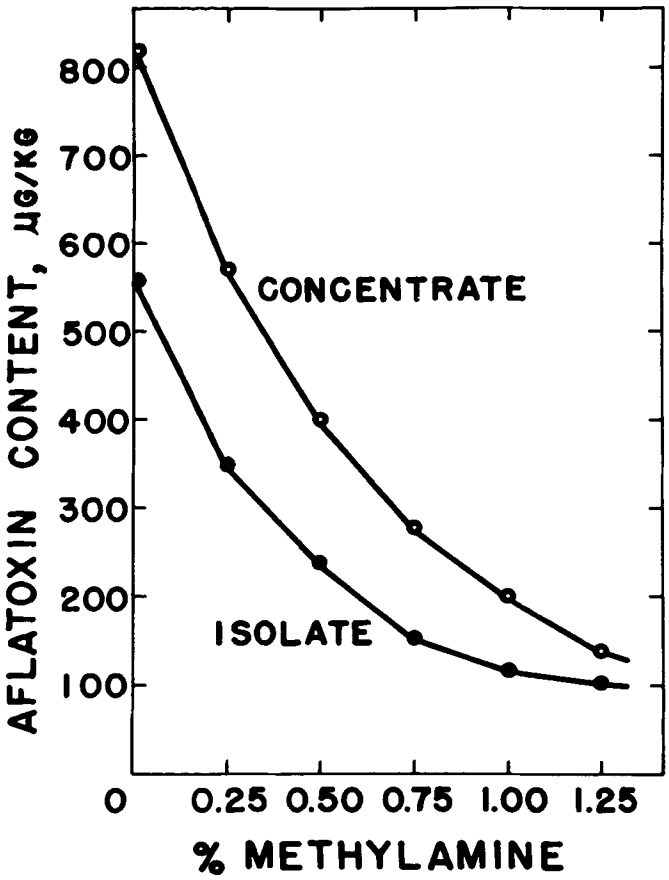

FIG. 3. Effect of methylamine concentration in process water on the aflatoxin content of peanut protein concentrate and isolate.

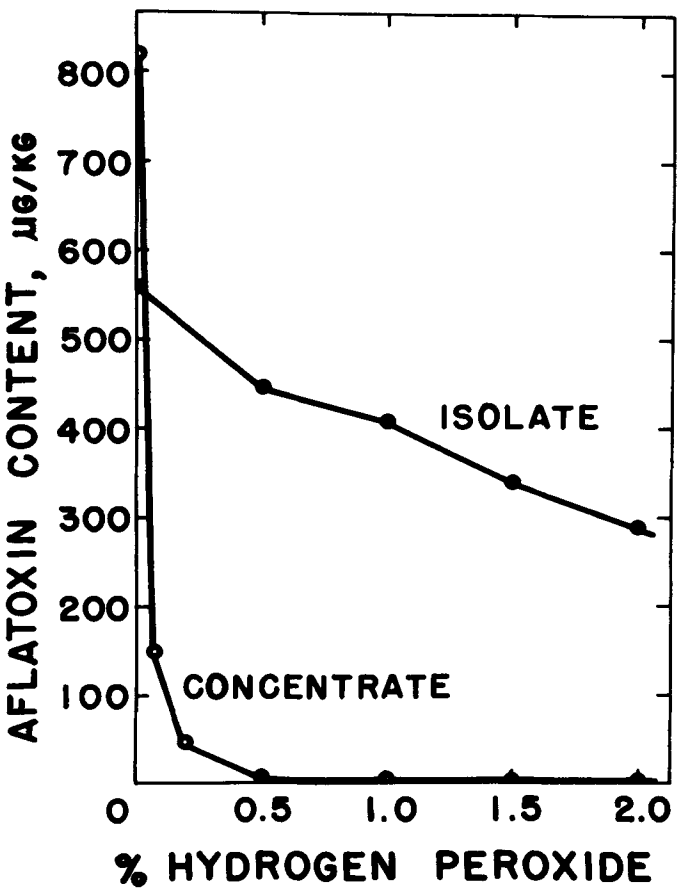

FIG. 4. Effect of hydrogen peroxide concentration in process water on the aflatoxin content of peanut protein concentrate and isolate.

of forming from aflatoxins other products having adverse physiological activity. Some of the disadvantages are: (a) added cost for additional processing and special equipment, (b) difficulty of solvent recovery, and (c) difficulty of removing residual acetone completely from the products which is necessary to avoid undesirable flavor problems.

Effect of Aqueous Isopropyl Alcohol

The effect of aqueous isopropyl alcohol on the extraction of aflatoxins in the protein concentrates and isolates prepared from contaminated peanuts is reported in Figure 2. Under the conditions tested, 80% isopropyl alcohol eliminated the aflatoxins from both concentrates and isolates to below levels of detection. Isopropyl alcohol concentrations below 80% in process water reduced aflatoxins significantly but did not eliminate them completely. Also, 100% isopropyl alcohol proved to be a rather poor solvent for this purpose.

The effect of isopropyl alcohol concentration and temperature of extraction on removal of aflatoxins from defatted cottonseed and peanut meals was reported by Rayner and Dollear (20). They reported that an extraction temperature higher than 60 C was required to completely eliminate aflatoxins using 80% isopropyl alcohol. Since isopropyl alcohol-extracted fish protein concentrate is approved for food uses, this solvent may be advantageously used to produce protein concentrates or isolates from aflatoxin contaminated peanuts. However, at this point, the economics of using this solvent seem rather unfavorable due to its high cost and added equipment necessary for its recovery.

Effect of Methylamine

The effect of methylamine concentrations on the inactivation of aflatoxins in protein concentrates and isolates prepared by the aqueous extraction process is reported in Figure 3. The incorporation of methylamine in the process water effectively reduced the aflatoxin level of both concentrates and isolates. However, even at the highest concentration of methylamine tested (1.25% in process water), the destruction was far from complete in both products under the experimental conditions used.

It has been reported that the same concentration of methylamine was very effective in destroying aflatoxins in defatted peanut meal, but the reaction was carried out by cooking the contaminated peanut meal in a stirred reactor for 90 min at 100 C with the moisture level of meal adjusted to 30% (8). Such an extended reaction time at high temperature for effectiveness in destroying aflatoxins makes this chemical unsuitable for the aqueous extraction processing of contaminated peanuts.

Effect of Hydrogen Peroxide

The effectiveness of hydrogen peroxide in destroying aflatoxins under the conditions of the aqueous extraction processing of contaminated peanuts is summarized in Figure 4. Hydrogen peroxide treatment was not effective in achieving aflatoxin destruction under the conditions of the isolate process. This result does not quite agree with the reported results by Screenivasmurthy et al. (7) who claimed 97% destruction of aflatoxins with hydrogen peroxide. According to their work, a 10% aqueous suspension of a highly contaminated peanut meal was treated with hydrogen peroxide at pH 9 and 80 C for 30 min. Under these conditions, 0.5 ml of 6% hydrogen peroxide was required to destroy 10 µg of crystalline aflatoxin B_1. The peanut samples used in the present study contained about 26 µg aflatoxin (in 25 g samples). This would require about 1.3 ml of 6% hydrogen peroxide. Since the amount of hydrogen peroxide used in the present study was more than the

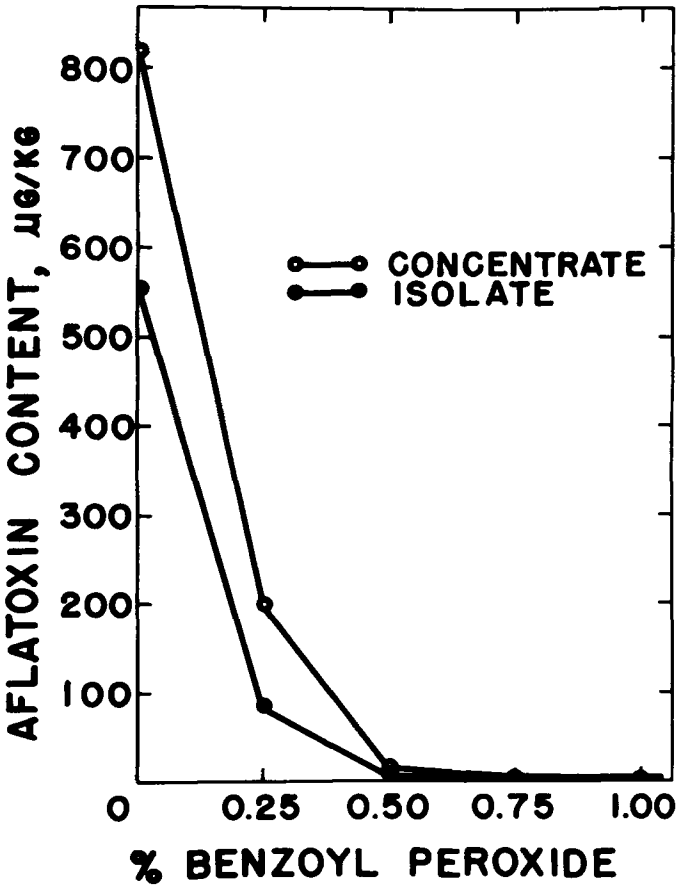

FIG. 5. Effect of benzoyl peroxide concentration in process water on the aflatoxin content of peanut protein concentrate and isolate.

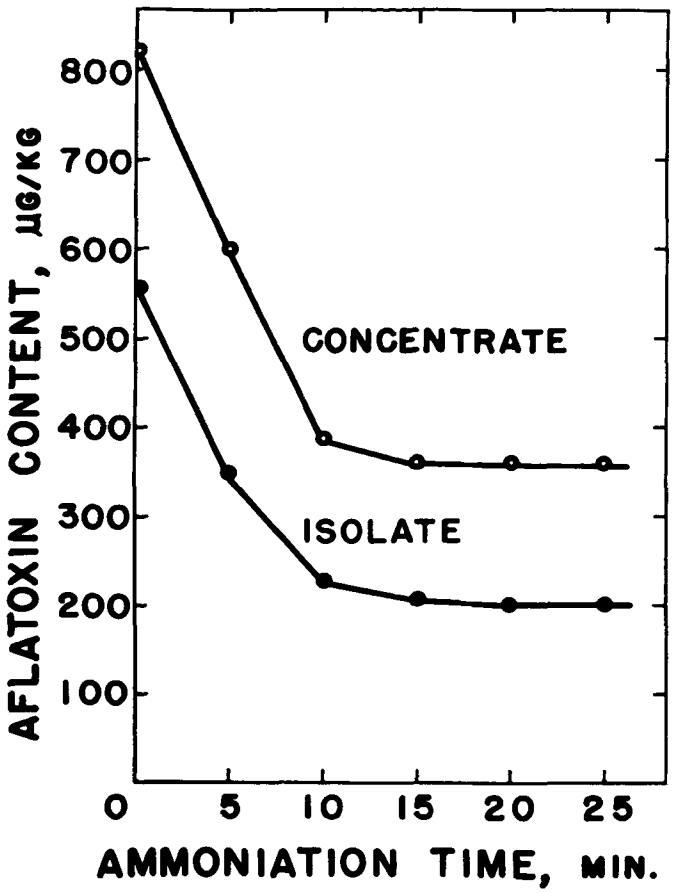

FIG. 6. Effect of ammoniation time on the aflatoxin content of peanut protein concentrate and isolate.

amount required on the basis of above-mentioned work, the failure to obtain aflatoxin inactivation would appear to be due to the reaction parameters such as temperature and, particularly, pH, since this chemical was effective in the concentrate process in which extraction is conducted at pH 4.

Hydrogen peroxide, as indicated in Figure 4, is a very effective chemical reagent in destroying aflatoxins in the concentrate process. Even at 0.5% hydrogen peroxide concentration, nearly 99% of the toxin was destroyed leaving only ca. 10 µg/kg toxins in the concentrates. The only difference between these two processes is the pH of the suspension at which hydrogen peroxide was added to react with aflatoxin. It would therefore appear from the results that an acidic environment is a necessity at the time of initial reaction for the hydrogen peroxide to achieve maximum effectiveness in destroying aflatoxins.

Effect of Benzoyl Peroxide

Figure 5 shows the effect of benzoyl peroxide on the aflatoxins during aqueous extraction processing of contaminated peanuts. For the isolate process, benzoyl peroxide appears to be more effective than hydrogen peroxide in reducing the aflatoxin content in the protein isolates. The use of 0.5% or higher concentrations of benzoyl proxide reduced the total aflatoxin levels below 30 µg/kg. Benzoyl peroxide was as effective as hydrogen peroxide for the concentrate process. Since benzoyl peroxide has been approved as a bleaching agent in the manufacture of certain cheeses (21) and wheat flour (22), this reagent has a potential for use in inactivation of aflatoxins in peanuts. However, benzoyl peroxide was not readily soluble in the aqueous suspensions. This may pose a problem in using this reagent in the aqueous extraction process. Also excess benzoyl peroxide may react with proteins and lipids resulting in undesirable flavors (23).

Effect of Ammonia Gas

The effect of ammonia treatment of aqueous suspensions of contaminated peanuts on the destruction of aflatoxins is summarized in Figure 6. For this study, ammonia gas was introduced into the suspension at atmospheric pressure at a rate of ca. 100 cc/min/100 ml as measured by a flow meter. The results indicate that significant reduction of the aflatoxin content of both isolates and concentrates occurred for the first 10 min of treatment. However, the reduction rate gradually leveled off as the reaction time proceeded further.

Gardner et al. (9) reported that both elevated temperature (90-125 C) and ammonia gas pressure (40-50 psig) were critical vairables for inactivation of aflatoxins. Failure to achieve satisfactory destruction of aflatoxins under the conditions of the aqueous extraction process with ammonia gas may very well be due to both the low reaction temperature and low gas pressure used in this study. Therefore it may be concluded that ammoniation would not be an effective method to destroy aflatoxins in peanuts as long as the aqueous extraction process is carried out under atmospheric pressure and at relatively low extraction temperature.

Effect of Sodium Hypochlorite

The effect of sodium hypochlorite concentration on the aflatoxin content of peanut protein concentrate and isolate is shown in Figure 7. Sodium hypochlorite was the most effective chemical reagent found for destroying aflatoxins during the aqueous extraction process. For both the concentrate and isolate processes, 0.2% sodium hypochlorite effectively reduced aflatoxin content of protein products to a barely detectable level. Fishbach and Campbell (24) have

demonstrated, by biological tests, the destruction of the toxicity of aflatoxins by sodium hypochlorite.

Based on the foregoing information, it appears to be technologically possible to reduce the aflatoxin levels in protein concentrates or isolates prepared from contaminated peanuts to within acceptable levels by carefully utilizing certain chemicals under proper aqeuous extraction processing conditions. In view of the discovery in recent years that myocotoxins are much more widely distributed in foodstuffs than previously suspencted and the urgent need for larger food supplies in the future to feed the world's rapidly expanding population, technology such as this for salvaging all available food materials will be of vital importance.

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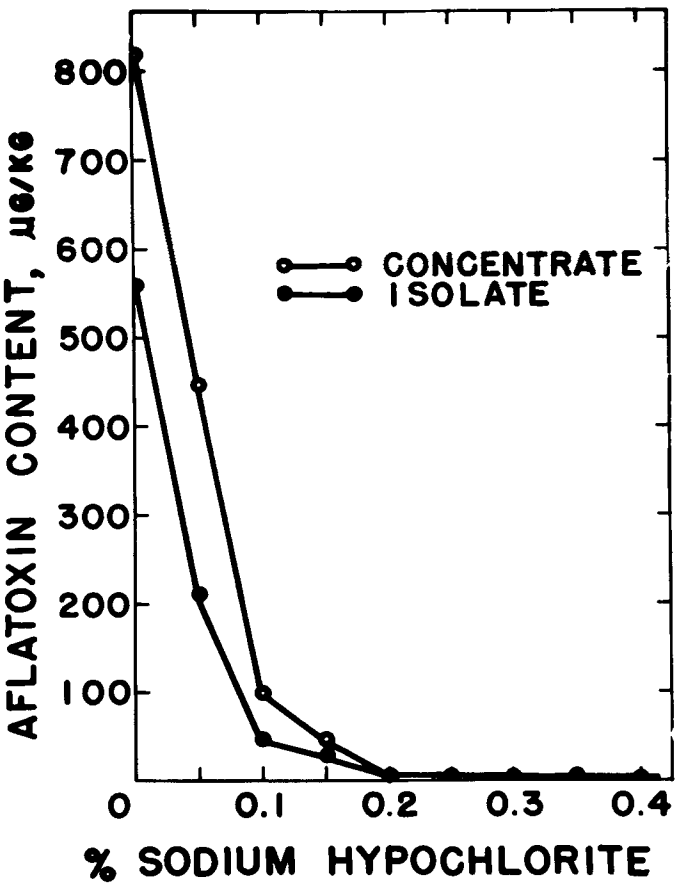

FIG. 7. Effect of sodium hypochlorite concentration in process water on the aflatoxin content of peanut protein concentrate and isolate.

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