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Reduction of aflatoxins in peanut meal by extrusion cooking in the presence of nucleophiles

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ABSTRACT

This study explored the feasibility of degrading aflatoxins in contaminated peanut meal by extruding in the presence of calcium chloride with lysine and methylamine. A 2 × 2 × 3 experiment (moisture, pH and nucleophile) was designed to screen for a nucleophile to use in a study of extrusion conditions to degrade aflatoxins in peanut meal. The nucleophile was mixed with peanut meal at 2 g/100 g protein level and the samples "spiked" with aflatoxin standards. They were extruded using a single screw extruder, and aflatoxins quantified by HPLC.

The presence of calcium chloride impeded the degradation of aflatoxins by extrusion. However, methylamine and lysine showed comparable efficacies to mediate aflatoxin reduction. There were significant ($p \leq 0.05$) interactions between moisture and pH, as well as moisture and temperature. Contour plots from regression models ($R^2 = 0.85$) showed a bimodal effect of moisture on aflatoxin degradation. Extrusion cooking reduced aflatoxins from an initial 417.72 μg/kg to 66.87 μg/kg (i.e. 84% reduction) in the peanut meal.

1. Introduction

The presence of aflatoxins in food constitutes a health hazard because of their suspected role in the etiology of liver cancer and other health complications in humans (IARC 1987, Groopman, Cain, & Kensler, 1988). To reduce the risk of exposure to the toxins through ingestion of contaminated food, several methods to decontaminate peanuts have been suggested. They include physical, microbiological, and chemical approaches (Ellis, Smith, & Simpson BKand Oldham, 1991; Rustom, 1997) but the chemical methods are particularly attractive because of their rapid execution.

The most effective chemical agents used to mediate the degradation of aflatoxins have been the nitrogen bases, especially ammonia and methylamine (Dollear, Mann, Codifer, Jr, Gardner, koltun, Vix, 1968; Mann, Codifer, Jr, Kotun, Dollear, 1970; Park, Lee, Price, & Pohland, 1988). The efficacies of these reagents in degrading aflatoxins depend on treatment variables such as temperature, time, moisture, and pressure. In the presence of excess moisture, aflatoxins B_1 is believed to undergo a hydrolytic opening of the lactone ring to form a terminal carboxylic acid (β-keto-acid), which undergoes heat driven decarboxylation to the less toxic aflatoxins D_1 (Bassapa & Shantha, 1996). Autoclaving of moist peanut meal completely destroyed aflatoxins (Coomes, Crowther, Feuell, & Francis, 1966).

Treatments that provide high temperatures and pressures similar to or higher than autoclaving (as in extrusion cooking) have shown some promising results. Giddey, Brandt, and Bunter (1977) reduced aflatoxins B_1 concentration in peanut cake from 2000 μg/kg to less than 50 μg/kg by mixing with a combination of methylamine (0.5 g/100 g) and calcium hydroxide (2 g/100 g) and extruding at 11–25 g/100 g dry matter moisture content, and 100–110 °C. Grehaigne, Chouvel, Pina, Graille, and Cheftel (1983) attained 87% degradation of aflatoxins by co-extruding contaminated peanut meal with ammonium hydroxide in a twin-screw extruder. The high shear and pressures attained in the extruder as well as the viscous dissipation of mechanical energy as heat are thought to provide a feasible bio-reaction environment to degrade aflatoxins.

In this study it is hypothesized that extrusion cooking of contaminated peanut meal in the presence of lysine might have the potential to reduce aflatoxin levels, similar to that reported for methylamine and ammonium hydroxide by Giddey et al (1977) and Greghaigne et al (1983) respectively. In a preliminary study (Saalia & Phillips, 2010) it was observed that indeed lysine and methylamine showed comparable efficacies and kinetic rate constants (k) in degrading aflatoxins in aqueous phosphate buffers. However in the presence of a food matrix, aflatoxins may bind to the macromolecules

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and get protected from structural degradation during processing (Tabata et al., 1992). To be able to substantially degrade aflatoxins under the circumstances, there is the need to overcome the forces of association with the macromolecules. This phenomenon was demonstrated by chemisorption of aflatoxin B_1 to HSCAS (Grant & Phillips, 1998). Calcium chloride will be used in this study in an attempt to reduce the binding of aflatoxins to the macromolecules in peanut meal and make them amenable to degradation reactions during extrusion cooking.

2. Materials and methods

Experiments were designed to determine (a) the efficacies of methylamine and lysine (b) the role of pH (c) and the influence of calcium chloride on aflatoxin degradation in partially defatted roasted peanut meal during extrusion.

2.1. Design of experiments

A full factorial design was replicated using moisture (20 g and 35 g/100 g DMB), pH (7.5 and 9.5) and nucleophile (lysine, methylamine and control) as variables were run to compare the efficacies of the nucleophiles on the reduction of aflatoxins during extrusion cooking. Following this, one nucleophile (lysine) was selected for use in a response surface study (Box-Behnken, 1960) to determine the optimum conditions to reduce aflatoxins during extrusion cooking. By their nature, Box-Behnken designs are replicated (usually 3 times) at the centre points to allow for a more uniform estimate of the prediction variance over the entire design space. Table 1 shows the variables and the levels as well as the replicated center points used in the study. For all experiments, partially defatted peanut meal (obtained from Seabrook Enterprises Inc., Sylvester, GA) was autoclaved to denature the proteins in order that high moisture levels of the experimental region (35 g/100 g DMB) could be attained without forming a plastic mass.

2.2. Sample preparation and extrusion cooking

The nucleophile (lysine and methylamine) was mixed with peanut meal at 2 g/100 g of the protein content. The meal moisture and pH were adjusted with 0.1 mol equiv./L sodium hydroxide solution. The samples were artificially contaminated ("spiked") with aflatoxins standards (Sigma Chemical Co, St. Louis, MO) to 200 μg/kg for AFB_1 and AFG_1 and 60 μg/kg forAFB_2 and AFG_2 and then thoroughly mixed under subdued lights. They were extruded using a pilot scale single screw extruder (Wayne Machine & Die Co, Totowa, NJ) equipped with a screw of 5:1 compression ratio, a 4 mm circular die and was run at the maximum speed of 135 rpm. The barrel temperature settings for the two zones closest to the die were varied from 125, 150, and 175 °C, while the feed port zone was left at ambient temperature. The extruder was choke-fed, and extrudates collected when steady state had been attained.

Table 1
Variables and their levels used in the Box Benkhen design.

Coded Values			Actual values		
Moisture	Temperature	pH	Moisture (g/100g)	Temperature (°C)	pH
+1	+1	0	35	175	7.4
+1	−1	0	35	125	7.4
−1	+1	0	20	175	7.4
−1	−1	0	20	125	7.4
0	0	0	27. 5	150	7.4
+1	0	+1	35	150	8
+1	0	−1	35	150	6.7
−1	0	+1	20	150	8
−1	0	−1	20	150	6.7
0	0	0	27. 5	150	7.4
0	+1	+1	27. 5	175	8
0	−1	+1	27. 5	125	8
0	−1	−1	27. 5	125	6.7
0	+1	−1	27. 5	175	6.7
0	0	0	27. 5	150	7.4

Codes refer to minimum (−1), midpoint (0) and maximum (+1) values of the variable.

2.3. Extrusion of peanut meal with calcium chloride

Calcium chloride was mixed at 0.1 g/100 g by weight to the aflatoxin contaminated peanut meal, and extruded as described above at the same conditions of pH and Temperature. However, the samples that had moisture at 20 g/100 g behaved too dry when mixed with calcium chloride and were therefore adjusted to 25 g/100 g to facilitate extrusion in the single screw extruder. The effects of calcium chloride on aflatoxin reduction in the peanut meal at varying moisture, pH and temperature were examined by determining residual aflatoxins in the extrudates.

2.4. Aflatoxin losses through steam flash off

To make certain that any observed reduction in aflatoxins was not due to losses through steam at the die, a cylindrical cold jacket apparatus (50 cm long, 10 cm diameter) with its inner surface lined with dry adsorbent (Whatman) paper was used to trap steam from the die. The cold jacket was fixed to the die plate such that flashed off steam condensed on the absorbent paper, while extrudates dropped through a slit (3.0 cm wide along the length of the jacket) into a collecting tray. After each extrusion run the wet absorbent paper was transferred into a black plastic bag for later analysis and quantification of aflatoxins.

2.5. Aflatoxin extraction and analyses

The procedure described by Clavero et al., 1993 for extraction of aflatoxins in peanuts was followed. Twenty-five grams (25 g) of the sample and 5 g sodium chloride were weighed into an explosion proof blender. Methanol/water (70/30 v/v), was added (125 mL) and blended at high speed for 2 min, then filtered (Whatman 2V-fluted paper) and diluted 1:5 with the solvent mixture. Fifteen (15) mL of the diluted filtrate was pipetted into 30 ml filtered (Millipore filter, type GN 0.2μ) deionised water. Fifteen (15) ml of the sample was passed through aflatest-P affinity column (Vicam, Watertown MA) which contains monoclonal antibodies that bind aflatoxins to the column packing material. The column was washed with 10 mL of filtered deionised water, and then the aflatoxins eluted with 1 mL methanol into a silanized amber vial. The contents were dried under nitrogen, and stored at −18 °C until analyzed using HPLC (Waters 2690 Separation module, Waters Corporation, Milford, MA USA)) procedures with fluorescence detection.

2.6. HPLC analyses and quantification

The sample was redissolved in 1 mL methanol, and injected (20 μL) onto the column (Econosphere $C_{18,}$ 5μ, 250 × 4.6 mm; Alltech Associates Inc., Deerfield, IL). The chromatographic procedure involved an isocratic elution, using a mobile phase of water-methanol-acetonitrile (60:25:15, v/v/v) at a flow rate of 1 mL/min (Waters 2690 separations module, Waters Corporation, Milford, MA USA). The column was maintained at 60 °C, and detection of aflatoxins G_1 and B_1 was enhanced by post column derivitization using freshly prepared saturated aqueous iodine (Shepherd & Gilbert, 1984), run at a flow rate of 0.3 mL/min to mix with the eluate