LWT - Food Science and Technology 44 (2011) 1496–1501

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Degradation of aflatoxins by extrusion cooking: Effects on nutritional quality of extrudates

Firibu K. Saalia [a,*], Robert D. Phillips [b]

[a] Department of Nutrition and Food Science, University of Ghana, Legon, Accra, Ghana
[b] Department of Food Science and Technology, University of Georgia, Griffin, GA 30223, USA

ARTICLE INFO

Article history:
Received 18 February 2010
Received in revised form
24 January 2011
Accepted 26 January 2011

Keywords:
Aflatoxins
Extrusion
Available lysine
In-vitro digestibility

ABSTRACT

Extrusion of artificially contaminated food is reported to degrade aflatoxins to varying degrees depending on the extrusion conditions. This work sought to determine the (1) efficacy of extrusion cooking in destroying naturally contaminated peanuts and (2) nutritional quality of decontaminated peanut meal. Naturally contaminated peanut meal was extruded by varying the moisture (20, 28, 35 g/100 g), pH (7.5, 9.5) and extruder die diameter (2.5, 3, 3.5, 4.0 mm). Aflatoxins levels in the extrudates were determined using HPLC procedures, and the nutritional quality was assessed using *in-vitro* methods. The highest aflatoxin reduction in naturally contaminated peanut meal was 59% at feed moisture content of 35 g/100 g. Higher (91%) reduction was achieved in the artificially contaminated peanut meal at moisture of 20 g/100 g. *In-vitro* protein digestibility and Fluorodinitrobenzene (FDNB)-available lysine of the extrudates were not significantly different from non-extruded peanut meal. Extrusion conditions for aflatoxin reduction did not adversely affect protein nutritional quality.

1. Introduction

Extrusion cooking is a processing technology that involves pushing a granular food material down a heated barrel and through an orifice by a rotating, tight fitting Archimedean screw. The shear forces created by the rotating action of the screws, together with frictional, compressive and pressure forces provide the necessary environment for rapidly cooking and transforming the food into visco-elastic melt. Extrusion cooking is an efficient, high temperature short time process, and it is used to produce a wide variety of foods and ingredients. It has also been used as a kind of bioreactor to decontaminate aflatoxins in peanuts (Grehaigne, Chouvel, Pina, Graille, & Cheftel, 1983), glucosinolates in rapeseeds (Barrett, Klopfenstein, & Leipold, 1997), and canavanine in jack beans (Tepal, Castellanos, & Larios, 1994) In a previous study (unpublished) we demonstrated that extrusion cooking of artificially contaminated peanut meal achieved 84% reduction in total aflatoxins content. The feed moisture, barrel temperature and the die diameter (all of which impact on the throughput and consequently the residence time) were the variables that influenced aflatoxins reduction during extrusion cooking.

To destroy or inactivate aflatoxins, the extrusion cooking conditions need to be severe (high shear, high temperature, and the right pH) in order to provide the necessary environment in the barrel. Such treatments to destroy or inactivate aflatoxins in peanuts may affect essential nutrients and compromise the nutritional quality of the food product. Alonso, Aguirre, and Marzo (2000) reported that extrusion processing of faba and kidney beans improved protein and starch digestibilities. Phillips (1989) and Camire (1991) explained that extrusion cooking conditions cause physical and chemical transformations that may impact on the nutritional quality of the extruded material. Such transformations include protein cross-linking (Phillips, 1989; Stanley, 1989), isopeptide bonding (Burgess & Stanley, 1976) or amino acid racemization (Friedman, 1999). In order to facilitate aflatoxin destruction, the medium is sometimes modified by alkaline treatment to raise the pH (Giddey, Brandt, and Bunter 1977). Such process conditions might also result in the racemization of amino acids as well as promote lysinoalanine formation (Friedman, 1994, 1999; Kato, Tanaka, Lee, Matsudomi, & Kobayashi, 1987). These reactions result in the reduction of protein digestibility and amino acid bioavailability. The objectives of this study were to determine the efficacy of extrusion cooking in decontaminating naturally contaminated peanuts, and the protein nutritional quality of the decontaminated product.

* Corresponding author.
E-mail address: fsaalia@ug.edu.gh (F.K. Saalia).

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doi:10.1016/j.lwt.2011.01.021

2. Materials and methods

2.1. Naturally contaminated peanut meal

Defatted naturally contaminated peanut meal was donated by Cargill Peanut Products, Dawson, GA, USA. It had a crude fat content of less than 3 g/100 g and crude fiber of 12 g/100 g, because it was made by crushing the seeds with the shells and then solvent extracted. The moisture was adjusted to three levels (20, 30 and 40 g/100 g) and the pH to two levels (pH 7.5–9.5). Lysine (purchased as L-lysine monohydrochloride, ICN Biomedicals, Inc, Aurora, OH, USA) was added to the peanut meal at 2 g/100 g of the peanut protein content, and thoroughly blended and adjusted to pH 8, before extrusion cooking, using four different die diameters (2.5, 3.0, 3.5, 4.0 mm) at extruder barrel temperatures of 120, 130 and 140 °C. Clean, roasted peanut meal was artificially contaminated with aflatoxin (Sigma Chemical Co, St Louis, MO, USA) at 200 μg/kg for each of B_1 and G_1 and 60 μg/kg for each of B_2 and G_2, then adjusted to 20 g/100 g moisture and extruded (a treatment condition that had been pre-determined to achieve high aflatoxins reduction).

2.2. Extrusion cooking of contaminated peanut meal

The Wayne single screw extruder (Yellow Jacket, Wayne Machine and Die Co, Totowa, NJ, USA) equipped with a constant pitch screw with increasing root diameter, and a compression ratio of 1:5, was run at 135 rpm screw speed. The barrel temperature settings for the two zones closest to the die were varied from 120, 130, or 140 °C, while the feed port zone was left at ambient temperature. The extruder was choke fed, and samples were collected when steady state conditions were attained. They were cooled and stored in dark plastic bags at −18 °C until they were analyzed for aflatoxins.

2.3. Aflatoxins extraction and quantification

The aflatoxins extraction procedure was as described by Clavero, Hung, Beuchat, and Nakayama (1993) and it was done in triplicate for each sample. Twenty-five (25) grams of sample and 5 g sodium chloride were weighed into a high speed, explosion proof blender. Methanol/water (70:30, mL:mL) was added (125 mL), and blended at high speed for 2 min. The slurry was filtered (Whatman 2V fluted paper) and diluted 1:5 with methanol/water (70:30). Fifteen (15) mL of the diluted extract was pipetted into 30 mL filtered water (Millipore filter, type GN 0.2 μm). Fifteen (15) mL of this was passed through the Aflatest-P affinity column (Vicam, Watertown, MA, USA) which contains monoclonal antibodies that bind the aflatoxins. The column was washed with filtered deionized water (10 mL) and the aflatoxins eluted from the column with HPLC-grade methanol into a silanized amber vial. Each sample was extracted using the above outlined procedure in triplicate. Quantification of aflatoxins was done using HPLC procedures with fluorescence detection (Waters Corporation, Milford, MA, USA).

2.4. HPLC analyses of aflatoxins

HPLC analysis was carried out using the Waters 2695 Separation module, equipped with an Econosphere C_{18}, 5μ, 250 × 4.6 mm reverse phase column (Alltech Associates Inc., Deerfield, IL, USA) maintained at 60 °C. The mobile phase consisted of water–methanol–acetonitrile (60:25:15, mL/mL/mL) and was run at a flow rate of 1 mL/min. To enhance the fluorescence of aflatoxins B_1 and G_1, post-column derivatisation with freshly prepared saturated aqueous iodine was employed (Shepherd & Gilbert, 1984). Aflatoxin detection was by the Waters 474 florescence detector (Waters Corp, Milford, MA, USA), set at 435 nm EM and 365 nm Ext. Data acquisition and processing were performed using the Waters Millennium software version 3.2 (Waters Corp, Milford, MA, USA). Peak areas were used to determine aflatoxin concentrations in the samples by reference to calibration curves obtained from a mixed aflatoxins standard (Sigma Chemical Co, St Louis, MO, USA) under identical conditions of HPLC. This method has previously been determined to have a detection limit of about 1 μg/kg both for the standard solutions and for the peanut (extrudate) extracts. The recoveries for aflatoxins ranged between 80 ± 2.5 μg/100 μg and 110 ± 5.6 μg/100 μg.

2.5. Nutritional quality evaluation

2.5.1. Nitrogen determination

Nitrogen determination was by the modified Dumas combustion method (LECO FP-2000 Protein/Nitrogen analyzer, LECO Corporation, Warrendale, PA). Ground samples were weighed (1.5 g) into a porcelain sample holder (boat) for introduction into the combustion chamber set at 1050 °C. The percent nitrogen obtained was multiplied by a factor of 5.46 to obtain the protein content of the peanut meal and extrudates.

2.5.2. Fluorodinitrbenzene (FDNB)-available lysine

The procedure of Carpenter (1960), as modified by Booth (1971) was followed. This involved reacting the peanut meal proteins with 1-fluoro-2, 4-dinitrobenzene (FDNB) in absolute ethanol and $NaHCO_3$ at room temperature for 2 h, followed by hydrolysis of the ϵ-dinitrophenyl protein (DNP-protein) in 8.1 mol/L HCl for 16 h, extraction with diethyl ether to remove interfering compounds, and measurement of ϵ-dinitrophenyl lysine (DNP-lysine) by absorbance at 435 nm. A blank value was obtained by treatment of a paired hydrolyzed sample with methoxycarbonyl chloride, followed by extraction with diethyl ether. The standard factor of 1.09 was used to correct for the loss of ϵ-dinitrophenyl lysine during acid hydrolysis (Booth, 1971).

2.5.3. In-vitro digestibility

The (pH drop) method of Hsu, Vavak, Satterlee, and Miller (1977) as modified by Satterlee, Marshall, and Tennyson (1979) was followed. A suspension (50 mL) of the sample (6.25 mg protein/mL) in distilled water was adjusted to pH 8.0 (using 0.1 mol equi/L NaOH) while stirring in a water bath maintained at 37 °C. The multienzyme solution (1.6 mg trypsin, 3.1 mg chymotrypsin, and 1.3 mg peptidase/mL (Sigma Chemical Co, St Louis, MO, USA)) adjusted to pH 8.0 was added to the sample suspension with constant stirring at 37 °C. After adding 1 mL of the protease solution (7.95 mg/mL, from *Streptomyces griseus*) at 10 min of the reaction, the tubes were transferred to a water bath maintained at 55 °C. After 19 min from the start of the reaction, the sample was transferred back to the 37 °C water bath, and the pH measured exactly 20 min from the start of the reaction.

2.5.4. Amino acid profile analyses

This followed the Waters Accq.tag precolumn derivatization method for amino acids determination. The procedure involved acid hydrolysis of sample proteins in 6 mol equi/L HCl for 24 h at 110 °C. After diluting with an internal standard and filtering, a small aliquot was used for derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Waters AccQ*Flour reagent, AQC). The AQC-tagged amino acids were analyzed by reverse phase HPLC using fluorescence detection (Waters 474 Waters Corp, Milford, MA, USA) with excitation at 250 nm and emission measured at 395 nm.