Pak. J. Biochem. Mol. Biol. 2011; 44(2): 56-59

Degradation of aflatoxins by roasting in contaminated peanuts

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Abstract: Contamination of peanuts with aflatoxins is one of the main factors that destroy the quality of the products. Aflatoxins are potent sources of health hazards to both human and animals and a great deal of effort has been made to completely eliminate the toxin or reduce its content in foods. Although prevention is the most effective intervention, heat has been used to inactivate aflatoxins in contaminated foodstuff. Nuts as general and especially peanuts are very sensitive commodity to aflatoxins contamination. In this study reduction of aflatoxins content in peanuts by roasting has been tested in a laboratory setting with aiming to suggest an optimal condition for the roasting. The artificially and naturally contaminated peanuts samples were treated by roasting at different temperatures. Although all treatment protocols showed some degree of aflatoxins degradation (ranging from 20% to 65%), roasting artificially contaminated samples at 120ºC for 120 min and 150ºC for 30-120 min caused substantial reduction of aflatoxins in samples. Treatment of naturally contaminated whole peanut kernels at 150ºC for 30 min significantly reduced level of aflatoxins contamination in samples. Degradation of aflatoxins was both time and temperature dependent. Roasting at 150ºC for 120 min degraded more than 95% of aflatoxin B_1 in peanuts. Naturally contaminated samples with aflatoxins were more resistant to degradation by roasting.

Keywords: Peanuts, roasting, aflatoxins, reduction.
Received: March 10, 2011 **Accepted**: May 15, 2011
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INTRODUCTION

Peanuts are used in the fabrications of sweet, candies, pastes and mainly as a raw material in oil production. The peanuts seed possesses a high nutritional and commercial value due to the presence of proteins, fatty acids, carbohydrates and fibers, in addition to vitamins, calcium and phosphorus[1]. Aflatoxins are difuranocoumarin derivatives and secondary metabolites produced by many strains of *Aspergillus flavus*, *Aspergillus parasiticus* and the rare *Aspergillus nomius*, which contaminate plants and plant products. They have been detected in various food commodities from many parts of the world and are presently considered as one of the most dangerous contaminants of foods and feeds[2]. Although 20 aflatoxins have been identified, only 4 of them, that is, the aflatoxins B_1, B_2, G_1 and G_2, occur naturally and are significant contaminants of a wide variety of foods and feeds. AFB_1 is classified by the International Agency of Research on Cancer as Group 1 carcinogen[3].

Several epidemiological studies have implicated AFs in the increased incidence of human gastrointestinal and hepatic neoplasms in Africa, the Philippines and China. AFB_1 also has been implicated in human liver cell carcinoma[4]. Recently, AFs outbreaks affecting a large geographical area and causing over 123 deaths were reported in Kenya[5]. In many developing countries, the humid atmospheric conditions result in unacceptable levels of aflatoxins in harvested peanuts, tree nuts, and other foods[6].

In the last decade prevention of mould contamination in some foodstuffs especially nuts became a public health concern. Any negligence in implementation of Good Agriculture Practice (GAP) regulations in cultivating, harvesting and storage of nuts including peanuts provides desirable conditions for invasion by toxicogenic strains of *Aspergillus flavus* which could result in the production of highly toxic aflatoxins[7]. With increasing knowledge and awareness of AFs as a potent source of health hazards to both human and animals, a great deal of effort has been made to completely eliminate the toxin or reduce its content in foods and feedstuffs to significantly lower levels. Although prevention is the most effective intervention, chemical, biological and physical methods have been investigated to inactivate AFs or reduce their content in foodstuff[8]. Cleaning, mechanical sorting and separation, thermal inactivation, density segregation, irradiation, ultrasound, solvent extraction and adsorption classified as physical methods[9-12]. Use of heat to inactivate AFs in contaminated food has been attempted. It has been reported that AFs in contaminated food can be degraded by heat treatment. Several investigators have observed that AFs are degraded by roasting[13-17]. The extent of destruction was depended on initial level of contamination, heating temperature, moisture content and duration of heating[8]. The toxin from olive oil was inactivated 65% at 250ºC for 10 min and at 200 ºC for 20 min caused less reduction. Even when the temperature was kept at 150ºC for 100 min, there was little loss of AFB_1[15].

It has reported that heat is effective in reduction of AFs in cottonseed meal. 74.8% of aflatoxins (B_1+B_2) were degraded by heating at 100ºC for 1 h at a moisture content of 30% in contaminated cottonseed meal while 32.7% degradation occurred in a similar meal containing 6.6% moisture, under similar condition. It has been suggested that the presence of moisture in foods help in opening the

lactone ring in chemical structure of AFB_1 to form a terminal carboxylic acid; which then undergoes a heat-induced decarboxylation [8]. It has been reported that different drying methods, up to 80ºC for 5.5 h, did not have any significant influence on lipid quality of pistachio nuts [18]. Heating has been successfully used for decomposition of deoxynivalenol, nivalenol and zearalenone in naturally contaminated barely and wheat in a time and temperature dependent trend[19].

In order to evaluate degradation of AFs by roasting, naturally and artificially contaminated peanuts were used in experiments. Roasting of peanuts has been used traditionally to preserve and increase shelf life of peanuts in Pakistan. However, no scientifically designed experiments documented the effect of roasting on reduction of AFs content in peanuts. In this study the efficacy of this traditionally used method is tested in a laboratory setting with aiming to suggest an optimal condition for the roasting.

MATERIALS AND METHODS

Sample collection and preparation

Peanuts samples were purchased from retail shops and local market. Effect of roasting on reduction of AFs contamination in peanuts was evaluated in three conditions; on artificially contaminated grounded peanut kernels, on naturally contaminated grounded peanut kernels and on naturally contaminated whole peanut kernels in shell. Details of samples groups are presented (Table 1). Peanut kernels were grounded by using mechanical grinder. In order to increase homogeneity of samples, grounded samples were sieved. Non contaminated samples were artificially contaminated with known concentrations of AFB_1 and AFB_2.

Table 1: Type of samples used in experiment

Sample	Type	AFs contamination
Group 1	Artificially contaminated Ground kernels	B_1 (250 ppb), B_2 (50 ppb)
Group 2	Naturally contaminated Ground kernels	B_1 (54 ppb), B_2 (6.2 ppb)
Group 3	Naturally contaminated Ground kernels	B_1 (85 ppb), B_2 (8.3 ppb)
Group 4	Naturally contaminated Ground kernels	B_1 (230 ppb), B_2 (16 ppb)
Group 5	Naturally contaminated Whole peanuts	B_1 (132 ppb), B_2 (17.4 ppb)
Group 6	Naturally contaminated Whole peanuts	B_1 (220 ppb), B_2 (20.5 ppb)

Roasting experiment

Based on preliminary experiments, treatment of samples at 90ºC, 120ºC and 150ºC were selected for roasting. Temperature above 150ºC showed undesirable effects on taste and color of peanut kernels. Roasting at temperature below 90ºC did not show substantial reduction of AFs. Samples were layered in an aluminum container and roasted for 30, 60 and 120 min at selected temperatures in an electrical oven. Known quantity of each sample was collected from oven at designated time and after cooling at room temperature the AFs contents were analyzed.

Chemicals

All the chemicals of analytical grade used in the present study were procured from BDH (Poole, England), Merck (Darmstadt, Germany) and Sigma Chemicals (ST. Louis, USA). Standards of aflatoxin B_1 (2.02 µg/ ml) and aflatoxin B_2 (0.500 µg/ ml) were purchased from Biopure (Tecknopark Tullin, Austria). Standard stock solutions of AFB_1 and AFB_2 of concentrations 1 µg/ ml each were prepared by diluting in benzene/ acetonitrile (98:2; v/v). These stock solutions were then stored at 4°C in refrigerator, wrapped in aluminum foil due to that aflatoxins gradually breakdown under UV light.

Determination of aflatoxins

Aflatoxins B_1 and B_2, were determined according to the method described by Soares and Rodrı´giez-Amaya [20]. Briefly, 50 g of each sample of hulls and kernels was extracted with 270 ml methanol and 30ml 4% potassium chloride. Samples were blended at moderate speed for 30 min and filtered, and 150ml of the filtrate was collected into a graduated cylinder. Next, 150ml 10% copper sulfate and 50ml diatomaceous earth were added, followed by moderate stirring and filtration. The filtrate was again recovered up to 150ml and transferred to a separation funnel, and toxins were extracted three times with 10 ml chloroform. The chloroform extracts were collected into a beaker and submitted to solvent evaporation in a water bath at 60°C. Extracts were re dissolved in 500µl chloroform and immediately submitted to thin-layer chromatography (TLC).

Final identification and quantification of aflatoxins were performed by one-dimensional thin-layer chromatography on pre coated silica gel plates (Merck). The plates were developed in a saturated chamber with chloroform/acetone (9:1, v/v). Aflatoxins spots were observed under long-wave ultraviolet light (k=366 nm) and determined by visual comparison with AFB_1 and AFB_2 standards prepared. Confirmatory tests for aflatoxins were carried out using trifluoroacetic acid[21].

Recoveries study

The recovery (percentage of standard added to sample that is recovered after extraction and clean up) of extraction method was determined by sample fortification. Fifty grams of milled peanuts was fortified one hour before extraction with a solution of AF in benzene: acetonitrile (98:1 v/v) at 1µg/ml for B_1 and B_2. The AF fortification solution was prepared in benzene: acetonitrile and used for quantification of analyte recovered after extraction.

RESULTS AND DISCUSSION

Degradation of AFs in peanuts samples by roasting is presented (Tables 2 & 3). Reduction of AFs contamination in artificially contaminated ground peanut kernels (group 1 samples) by roasting at 90, 120 and 150ºC is shown (Figures Ia & Ib). Although all treatment protocols showed some degree of AFs degradation, roasting samples at 120ºC for 120 min and 150ºC for 30-120 min caused substantial reduction on level of AFs in samples. About 90% of AFs in samples were degraded by roasting for 120 min at 150ºC.

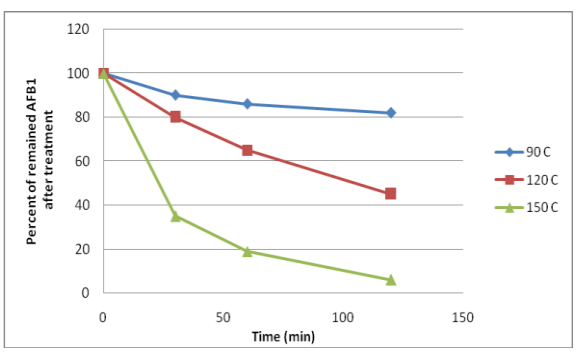

Figure 1a: Reduction of aflatoxins contamination on artificially contaminated ground peanut kernels by roasting at 90, 120 and 150ºC. Level of contamination of AFB_1 was 250 ppb.

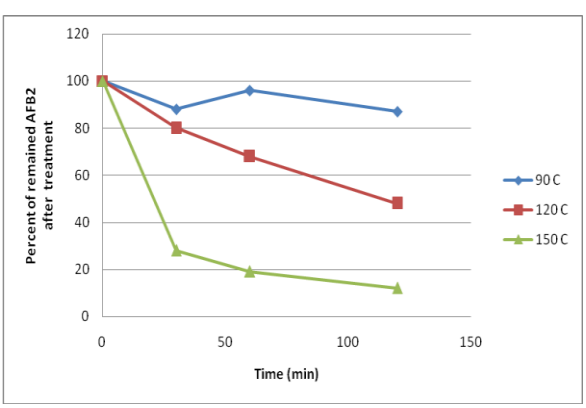

Figure 1b: Reduction of aflatoxins contamination on artificially contaminated ground peanut kernels by roasting at 90, 120 and 150ºC. Level of contamination of AFB_2 was 50 ppb.

Effect of roasting at 150ºC for 30 min on reduction of AFs in naturally contaminated ground peanuts kernels (sample groups 2-4) can be seen in (Table 2). Significant degradation of both AFB_1 and AFB_2 was observed in naturally contaminated samples by roasting at 150ºC for 30 min with out any noticeable change in taste of samples. However, even after roasting the AFs remained higher in naturally contaminated samples as compared to artificially contaminated samples.

Whereas roasting at 150ºC for 120 min changed physical appearance of peanuts to that of ‘‘burned’’ nuts and produced undesirable change in taste of peanuts. It appears that degradation of AFs is also dependent on their initial concentrations in samples. This might be due to availability of more active toxins for destruction during roasting. Treatment of samples at 120ºC for 2 h showed similar effects to roasting as at 150ºC for 30 min on reduction of AFs and minimum influence on color and taste.

Table 2: Effect of roasting at 150 ºC for 30 min on reduction of aflatoxins (AFs) in naturally contaminated ground peanut kernels (sample groups 2 - 4), as percent of AFs content remained after treatment.

Samples	AFs contamination	AFB_1 (%)	AFB_2 (%)
Group 2 AFB_1 AFB_2	54 ppb 6.2 ppb	65 -	- 62
Group 3 AFB_1 AFB_2	85 ppb 8.3 ppb	63 -	- 66
Group 4 AFB_1 AFB_2	230 ppb 16 ppb	23 -	- 32

However, heating samples for 2 h is practically difficult. Therefore roasting at 150ºC for 30 min was used as optimum condition for degradation of AFs in peanuts. Level of AFs has significantly reduced in naturally contaminated whole peanut kernels (sample groups 5 and 6) by treatment at 150ºC for 30 min Table 3. Roasting samples having 132 ppb initial level of AFB_1 at 150ºC showed level of AFB_1 remained 62%, while samples having 220 ppb initial level of AFB_1 showed level remained to 18% Table 3. It has been previously reported that destruction of AFs was depend on initial level of contamination, temperature, period of heating and moisture content[22]. However, no linear correlation was found between AFs content and post treatment degradation in peanuts. In a separate experiment, naturally contaminated whole peanut kernels were roasted at 150ºC for 30 min. Roasting whole peanut kernels having 20.5 ppb and 17.4 ppb initial level of AFB_2 at 150ºC for 30 min showed level remained 16% and 46 %, respectively Table 3.

Table 3: Effect of roasting at 150 ºC for 30 min on reduction of aflatoxins (AFs) in naturally contaminated whole peanuts (sample groups 5 and 6), as percent of AFs content remained after treatment.

Samples	AFs contamination	AFB_1 (%)	AFB_2 (%)
Group 5 AFB_1 AFB_2	132 ppb 17.4 ppb	62 -	- 46
Group 6 AFB_1 AFB_2	220 ppb 20.5 ppb	18 -	- 16

Roasting is one of the effective physical methods to remove or reduce AFs content in foodstuff, therefore, this will reduce possible health risks associated with AFs to the consumers. Roasting at different temperatures for different time intervals has substantially reduced AFB_1 levels in peanuts as can be seen in Table 2, 3 and Fig 1a. Degradation of AFB_1 and AFB_2 were both time and temperature dependent. Slightest effect showed by roasting at 90ºC for 30 min. However, more than 95% of AFB_1 and AFB_2 degradation was observed in artificially contaminated ground peanuts at 150ºC for 120 min (Figures 1a & 1b).

AFs content in naturally contaminated samples were more resistant to degradation by heat. The level of AFs remained in these samples were higher than artificially contaminated samples even after roasting at 150ºC (Tables 2 & 3). Heat supplied in the form of high energy microwaves can also destroy mycotoxins[23, 24]. It has been reported that reductions in the level of contaminated peanuts, ranged from 50-60% and 32-40% for AFB_1 and AFG_1 respectively, after microwave roasting at 0.7 kw for 8.5 min [16]. Average reduction has been reported in AFs content of peanuts, ranged from 45% to 83% for dry roasting[22]. Removal of AFs from unshelled peanuts by a traditional salt boiling process has also been reported[14, 16]. It was previously reported that boiling raw, unshelled peanuts with 5% sodium chloride water solution can reduce AFs up to 80%[14]. Presence of ionic salts will probably increase the extent of AFs degradation by heat.

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