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Effect of roasting on degradation of Aflatoxins in contaminated pistachio nuts

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Abstract

With increasing knowledge and awareness of aflatoxins (AFs) as potent sources 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. Although prevention is the most effective intervention, heat has been used to inactivate AFs in contaminated foodstuff. Nuts as general and especially pistachio nuts are very sensitive commodity to AFs contamination. In this study effect of roasting on reduction of AFs content in pistachio nuts has been tested in a laboratory setting with aiming to suggest an optimal condition for the roasting. Although all treatment protocols showed some degree of AFs degradation (ranging from 17% to 63%), roasting spiked samples at 120 °C for 120 min and 150 °C for 30–120 min caused substantial reduction of AFs in samples. Treatment of naturally contaminated whole pistachio kernels at 150 °C for 30 min significantly reduced level of AFs contamination in samples. Degradation of AFs was both time and temperature dependent. Roasting at 150 °C and 120 min condition degraded more than 95% of AFB1 in pistachio. However, the resulted product was not edible. AFs in form of naturally occurrence were more resistant to degradation with heat.

Keywords: Pistachio nuts; Roasting; Aflatoxins

1. Introduction

In the last decade prevention of mold 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 most of nuts including pistachio provides desirable conditions for invasion by toxicogenic strains of Aspergillus Flavus and this could result in the production of highly toxic aflatoxins (AFs). This contamination has been reported in Iran pistachio (FDCL, 2002). AFB1 is one of the most potent known hepatocarcinogen compounds (Massay et al., 1995). It is also a teratogen and a potent mutagen. Several epidemiological studies have implicated AF in the increased incidence of human gastrointestinal and hepatic neoplasms in Africa, the Philippines, and China. AFB1 also has been implicated in human liver cell carcinoma (Massay et al., 1995). However, no published data is available regarding extent of health risk to consumers due to presence of AFs in pistachio nuts.

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

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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 (Rustom, 1997). Cleaning, mechanical sorting and separation, thermal inactivation, density segregation, irradiation, ultrasound, solvent extraction and adsorption classified as physical methods (Basappa, 1983; Samarajeewa, 1991; Marth and Doyle, 1979; Muller, 1983). Although AFs are highly stable to dry heat up to temperatures below their thermal decomposition temperature (Betina, 1989), use of heat to inactivate AFs in contaminated food has been attempted. Some studies have indicated that AFs in contaminated food can be degraded by heat treatment. Several investigators have observed that AFs are degraded by roasting (Farah et al., 1983; Mahjoub and Bullerman, 1988; Pluyer et al., 1987; Staron et al., 1980; Waltking, 1971; Escher et al., 1973). The extent of destruction achieved was depended on initial level of contamination, heating temperature, moisture content and duration of heating (Rustom, 1997). The effect of temperature on AFs stability in olive oil was examined previously. A temperature of 250 °C for 10 min eliminated 65% of the toxin from olive oil. While 150 °C and 200 °C for 20 min caused little loss. Even when the temperature was held at 150 °C for up to 100 min, there was little loss of AFB1 (Mahjoub and Bullerman, 1988). Heat also reported to be effective in reduction of AFs present in cottonseed meal (Mann et al., 1967). It has been reported that level of AFs in contaminated peanuts could be reduced by heating (Mann et al., 1967). Dry roasting of mold damaged peanut kernels leads to an apparent loss or destruction of much of AFB1. It has been found that roasting of peanut kernels for 30 min at 150 °C reduced AFB1 by approximately 80%. Lee et al. reported average reduction in AFs content of peanuts ranged from 45% to 83% for dry roasting (Lee et al., 1969). AFB1 content has been reduced by 30–44% in peanuts by roasting for 30 min at 150 °C (Pluyer et al., 1987). Heating has been successfully used for decomposition of deoxynivalenol, nivalenol and zearalenone in naturally contaminated barely and wheat in a time-temperature-dependent trend (Yumbe-Guevara et al., 2003).

AFs contamination in pistachio nuts is varies drastically and very much depends on relative humidity and temperature during harvesting and storage period. It should be mentioned that at present neither Codex nor other international bodies has set limits for AFs contents in pistachio and countries have their own national limits with considerable variation. National standard limits for AFs contents in pistachio nuts in Iran for AFB1 and total AFs are 5 and 15 μg/kg, respectively.

According to a report published by Iran ministry of health 7926 pistachio samples were analysed using HPLC during March 2001 to March 2002 in Iran National Food and Drug Control Labs. The data indicated that 5390 (68%) samples were not contaminated (<limit of detection; LOD) with AFB1, 1324 (17%) samples contained AFB1 between LOD-2 μg/kg, 451 (5%) samples contained AFB1 between 2 and 10 μg/kg and 761 samples contained AFB1 higher than 10 μg/kg (FDCL, 2002). According to the report of Japanese Ministry of Health, among 2422 pistachio samples analysed during 1972–1989, 2339 (97%) samples were not contaminated (<LOD), 35 (1%) samples contained AFB1 between LOD-10 μg/kg and 48 (2%) samples contained AFB1 higher than 10 μg/kg (JECFA, 1998).

Although small portion of pistachio nuts may contain high concentration AFs, in order to examine effect of roasting on degradation of AFs, in this study highly contaminated pistachio nuts were used for experiments. Roasting of pistachio nuts has traditionally been used to preserve and increase shelf life of pistachio nuts in Iran. However, no scientifically designed experiments documented the effect of roasting on reduction of AFs content in pistachio nuts. 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.

2. Materials and methods

AFs standards for the experiments were purchased from Sigma Chemical Co. All solvents used for the experiments were of either HPLC or analytical grade. Aflatest immunoaffinity columns purchased from Vicam Company. Pistachio samples were obtained from market place and all were cultivated and collected in 2003. However, naturally contaminated pistachio samples obtained from Ministry of Health National Control Labs. A HPLC method using a C18, 25 cm × 4.6 mm, column running on a Waters 600 HPLC equipped with fluorescence detector was used for analysis of all samples. Mobile phase was water:methanol:acetonitrile (54:29:17) with a flow rate of 1 ml/min. The fluorescence detector was operated at excitation wavelength of 365 nm and emission wavelength of 435 nm. Post column derivatization with Kobra cell was used for detection and quantitation of samples.

3. Sample preparation

Effect of roasting on reduction of AFs contamination in pistachio nuts was evaluated in three conditions; on artificially contaminated ground pistachio kernels, on naturally contaminated ground pistachio kernels and on naturally contaminated whole pistachio kernels in shell. Details of samples groups are presented in Table 1. Ground samples were expected to have a more

Table 1
Type of samples used in experiments

Sample	Type	AFs contamination
Group 1	Artificially contaminated ground kernels	B1 (200 ppb), B2 (50 ppb)
Group 2	Naturally contaminated ground kernels	B1 (44 ppb), B2 (5.6 ppb)
Group 3	Naturally contaminated ground kernels	B1 (91 ppb), B2 (7.4 ppb)
Group 4	Naturally contaminated ground kernels	B1 (213 ppb) and B2 (12 ppb)
Group 5	Naturally contaminated whole pistachio	B1 (144 ppb) and B2 (18.5 ppb)
Group 6	Naturally contaminated whole pistachio	B1 (235 ppb), B2 (21.9 ppb)

homogenate distribution of AFs. This was confirmed by HPLC analysis of ground sample prior to spiking. Ground pistachio kernels were prepared by grinding shelled pistachio nuts using mechanical grinder and then spiked with known concentrations of AFB1 and AFB2. In order to increase homogeneity of samples, ground samples were sieved using sieve number 12. Noncontaminated samples were used for preparing artificially spiked samples.

4. Roasting experiment

Based on preliminary experiments heating at 90 °C, 120 °C and 150 °C were selected for roasting experiments. Temperature above 150 °C showed undesirable effects on taste and color of pistachio kernels. Roasting in temperature below 90 °C did not show substantial effect on reduction of AFs. Samples were layered as a thin layer in an aluminum container and roasted for 30, 60 and 120 min at selected temperatures in an electrical oven. Identical portion of each sample removed from oven at designated time and after cooling to the room temperature was analysed for its AFs content.

5. Sample analysis

Fifty grams of ground samples and 500 g of whole kernel samples were removed from oven at 30, 60 and 120 min post experiment. Ground samples were mixed for 30 min with 5 g of sodium chloride, 300 ml MOH: H2O (80:20 v/v) and 100 ml *n*-hexane. Following extraction, suspension was filtered and 20 ml of filtrate diluted with 130 ml di-ionized water and filtered through micro filter and 75 ml of this was used for extraction with aflatest immunoaffinity column. Whole kernel samples ground and mixed with 750 ml water and then 125 g of the mixture was used for further extraction and clean up according to the above mentioned method. The aflatest column was pre conditioned with 10 ml PBS (2–3 ml/min) and then 75 ml of the sample extract was passed through the column (2–3 ml/min). Finally column was washed with 15 ml water. For AFs extraction from the column 0.5 ml and then 0.75 ml HPLC grade methanol passed through the column. Water was added to the column elute to the volume of 3 ml and 100 μl of this was analysed by HPLC. Heterogeneity of AFs distribution in pistachio nuts is a technical difficulty in experiments and even under laboratory conditions, complete homogeneity was not attained. This caused substantial variation in results. However, degradation of AFs in samples showed positive relation with increasing temperature and period of roasting.

LOD for AFB1 and AF total were 0.1 ppb and 0.4 ppb, respectively. Extraction recovery for AFs was found to be between 70% and 110%. Intra day %CV for experiments was between 9.7% and 11.8%. For quantitation of AFs in samples, a separate calibration curve was established for each AF. Triplicate samples were used for setting calibration curve, determining LODs and extraction recovery. The mean of these experiments was used for calculation.

6. Results

Effects of roasting on degradation of AFs in pistachio samples presented in Tables 2 and 3 and Fig. 1. Fig. 1 shows effects of roasting on reduction of AFs in artificially contaminated ground pistachio kernels (group 1 samples). Although all treatment protocols showed some degree of AFs degradation, roasting samples at 120 °C for 120 min and 150 °C for 30–120 min caused substantial reduction on level of AFs in samples. About 90% of AFs in samples were destroyed by roasting for 120 min at 150 °C. Table 2 shows effect of roasting at 150 °C for 30 min on reduction of naturally AFs contamination in ground pistachio kernels (sample groups 2–4). However, even after roasting samples at 150 °C remained AF in naturally contaminated samples was higher than that of artificially contaminated samples. Treatment at 120 °C for 2 h had similar effects to roasting at 150 °C

Table 2
Effect of roasting at 150 °C for 30 min on reduction of naturally AFs contamination in ground pistachio kernels (sample groups 2–4), as percent of original AFs content remaining after roasting

Sample	AFB1 (%)	AFB2 (%)
Group 2		
AFB1 (44 ppb)	66	
AFB2 (5.6 ppb)		63
Group 3		
AFB1 (91 ppb)	64	
AFB2 (7.4 ppb)		67
Group 4		
AFB1 (213 ppb)	24	
AFB2 (12 ppb)		33

Table 3
Effect of roasting at 150 °C for 30 min on reduction of AFs contamination on naturally whole contaminated pistachios (sample groups 5 and 6), as percent of original AFs content remaining after roasting

Sample	AFB1 (%)	AFB2 (%)
Group 5		
AFB1 (144 ppb)	63	
AFB2 (18.5 ppb)		47
Group 6		
AFB1 (235 ppb)	19	
AFB2 (21.9 ppb)		17

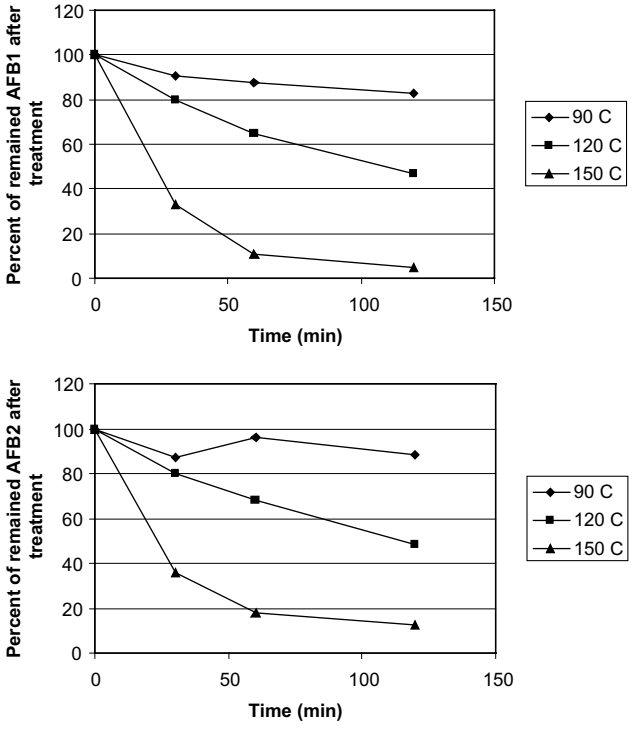

Fig. 1. Effect of roasting at 90, 120 and 150 °C on reduction of AFs contamination on artificially contaminated ground pistachio kernels. Level of contamination for AFB1 and AFB2 was 200 ppb and 50 ppb respectively.

for 30 min on reduction of AFs and minimum influence on color and taste. However, heating samples for 2 h is practically difficult. Therefore roasting at 150 °C for 30 min was used as optimum condition for degradation of AFs in pistachio nuts. Treatment of naturally contaminated whole pistachio kernels (sample groups 5 and 6) at 150 °C for 30 min significantly reduced level of AFs contamination in samples (Table 3).

7. Discussion

Roasting is one of the effective physical methods to remove or reduce AFs content in foodstuff. This will therefore reduce possible health risks from AFs to the consumers. As presented in Tables 2 and 3 and Fig. 1 roasting has substantially reduced AFB1 levels in pistachio. However, there is a significant difference between AFs decomposition in different treatment conditions ($p < 0.05$). Degradation of AFB1 was both time and temperature dependent. Roasting at 90 °C for 30 min showed slightest effect. However, 150 °C and 120 min condition degraded more than 95% of AFB1 in spiked ground pistachio (Fig. 1). Similar results were observed with AFB2 in contaminated pistachio nuts. Degradation of AFB2 was also time and temperature dependent (Fig. 1). Roasting ground pistachio kernels with naturally AFs contamination at 150 °C for 30 min produced significant degradation of both AFB1 and AFB2 without any noticeable change in taste of samples (Table 2). Roasting at 150 °C for 120 min changed physical appearance of pistachio nuts to that of "burned" nuts and produced undesirable change in taste of pistachio. It appears that degradation of AFs is also dependent on their initial concentrations in samples. This might be due to availability of more active materials for destruction during roasting. Roasting samples with 144 ppb initial level of AFB1 at 150 °C reduced level of AFB1 to 63% while in samples with 235 ppb AFB1 reduced to 19% of initial level (Table 3). This was also previously reported that destruction of AFs was depend on initial level of contamination, temperature, period of heating and moisture content (Lee et al., 1969). However, no linear correlation was found between AFs content and post treatment degradation in pistachio nuts. In a separate experiment whole pistachio kernels with naturally contamination of AFs were roasted at 150 °C for 30 min. Roasting whole pistachio kernels with initial level of AFB2 contamination of 21.9 ppb and 18.5 ppb reduced level of contamination to 17% and 47%, respectively (Table 3). AFs in form of naturally occurrence were more resistant to degradation with heat. Even after roasting samples at 150 °C remained AF in naturally contaminated samples was higher than that of artificially contaminated samples (Tables 2 and 3). Pluyer et al. was also reported that oven roasting at 150 °C for 30 min caused a 30–45% reduction of AFB1 in naturally contaminated peanuts. However, with artificially contaminated peanuts treated under the same conditions, destruction was 48–61% (Pluyer et al., 1987). This might be due to more easily availability of AFs in spiked samples for degradation by heat.

Using high temperature for roasting food commodities may produce harmful products. Therefore it is essential that, before conducting a full toxicological study on possible effects of degraded products, to use the lowest possible heating temperature for roasting pistachio in order to avoid producing possibly harmful by products. It is also predicted that roasting in high temperature may have some effects on nutritional contents

of pistachio nuts. Although in this study neither toxicological profile of degraded products nor effect of roasting on nutritional value of pistachio was investigated there are some reports on effect of drying and roasting on quality of pistachio nuts. It has been reported that different drying methods, up to 80 °C for 5.5 h, did not have any significant influence on lipid quality of pistachio nuts (Kashani Nejad et al., 2003). However, it has been also reported that the common method of salting and roasting pistachio nuts causes decrease in total available carbohydrates, in total free sugars and in total starches and dextrins. Similarly, free amino acids but not protein amino acids decrease (Kashani and Valadon, 1984). In order to reduce heat temperature and treatment period, it is suggested that co administration of some commonly used food additives with roasting may accelerate destruction of AFs in pistachio even in more gentle roasting condition. It has been reported that AFs has been removed from unshelled peanuts by a traditional salt boiling process (Pluyer et al., 1987; Farah et al., 1983). It was previously reported that boiling raw, unshelled peanuts with 5% sodium chloride water solution can reduce AFs up to 80% (Farah et al., 1983). Presence of ionic salts will probably increase the extent of AFs degradation by heat. In fact in Iran also most of pistachios are roasted using combination of heat and sodium chloride. Therefore, it is proposed that the effect of this combination may be examined on degradation of AFs in pistachio nuts.

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