Aflatoxins B_1 and B_2 Contamination of Peanut and Peanut Products and Subsequent Microwave Detoxification

A.K. Mobeen*, A. Aftab, A. Asif and A.S. Zuzzer

Food and Feed Safety Laboratory, Food and Marine Resources Research Centre, PCSIR Labs Complex, Karachi -75280, Pakistan

Abstract: The effectiveness of microwave heating has been evaluated for the detoxification of aflatoxins contaminated peanut and peanut products. The products comprise of various confectionery such as peanut brittle toffee, peanut brittle slabs of sugar and jaggery, roasted and salted peanut and peanut butter which were highly contaminated with aflatoxins B_1 ranging from 5 to 183µg/kg and aflatoxin B_2 ranging from 7 to 46.7µg/kg. The level of aflatoxins was determined and subsequently products were treated to microwave heating, which resulted in the reduction of aflatoxins content. The microwave cooking resulted in 50 to 60 % reduction in the levels of aflatoxins B_1, while B_2 was reduced to non-detectable limits.

Keywords: Aflatoxins, peanut products, microwave oven, detoxification.

INTRODUCTION

Mycotoxins are the secondary metabolites of fungi. Over 400 different mycotoxins have been isolated, but only a few of them have been of real concern in terms of human and animal health [1]. Aflatoxins are the most important group of mycotoxins produced by certain strains of the fungi *Aspergillus flavus* and *A. parasiticus*. Aflatoxin B_1 is one of the most potent naturally occurring hepatotoxin, teratogen, carcinogen and immuno-suppressive agent effecting both human being and animals [1]. It has been implicated in the etiology for liver cancer in conjunction with Hepatitis B virus in Pakistan as well as in other countries [2, 3]. Even small amounts of aflatoxins ingested over a period can prove to be extremely hazardous and can be aggravated with the poor nutritional status [4]. Kwashiorkor is caused by a protein deficient diet. Early symptoms of kwashiorkor include irritability and fatigue. As the condition continues, additional symptoms include slowed growth, weight loss, muscle wasting, generalized swelling, skin changes, enlargement of the liver and abdomen, and weakening of the immune system, leading to frequent infections [5]. Thus aflatoxin can be proved extremely hazardous if the poor nutritional diet is given to the human.

Human may get exposed to aflatoxins either directly by consuming contaminated nuts, cereals, fruits, vege-tables, etc., or indirectly by consuming milk from animals that have been fed aflatoxins contaminated feed, where aflatoxin B_1 or B_2 is converted into aflatoxin M_1 or M_2 and is secreted into the milk [4]. The problem of aflatoxins has been more commonly associated with peanut and corn as well as several other cereal grains and nuts [1, 6, 7]. Peanut and peanut products are highly susceptible to aflatoxin contamination either in the field or during storage, particularly in the hot and humid climatic conditions prevailed in a region like Pakistan [7]. Factors influencing aflatoxin production include high moisture and temperature, insect damage, relative humidity, etc. Agricultural commodities are stored in the tropical and sub-tropical regions, including Pakistan, are more prone to aflatoxin contamination partially due to the warm and humid climatic conditions, and partially due to the poor practices followed during harvesting, processing and storage.

Food processing, particularly cooking, affects levels of naturally occurring aflatoxins, depending upon the moisture content, type of matrix, natural composition and additives in food [8]. The previous studies have shown some destructive effects of various foods processing on the stability of added aflatoxins or naturally contaminated aflatoxins in food [8,9]. The important factors to the disintegration of aflatoxin are the matrix, physico-chemical characteristics and additives of the food [8]. Food processing destroys the aflatoxin to some extent whereas; general resistance to destruction is observed [8-10]. Various methods of cooking, *e.g.* frying, roasting, and conventional microwave baking, etc., have shown varying degree of destruction of aflatoxins [8, 10, 11]. Partial destruction of aflatoxin occurs in various stages of bread making, and it was proposed that this may be due to oxidation and hydrolysis phenomena [12].

The European Union (EU) permitted a maximum level of 4 ppb for total aflatoxins while according to Food & Drug Administration (FDA) a maximum tolerable level is 20 ppb in peanut [13, 14, 15]. In Pakistan, the use of peanut is increasing in

*Address corresponding to this author at the Food and Feed Safety Laboratory, Food and Marine Resources Research Centre, PCSIR Labs Complex, Karachi, Karachi -75280, Pakistan;
E-mail: fnfsafety@yahoo.com

confectionery, snack foods, sweet dishes and in the form of processed peanut butter. Small quantity of peanut products is consumed in the country. However, the long term accumulating toxic effects of aflatoxins cannot be ignored. Previous research work has shown high levels of aflatoxin in peanut [7], but no work has been done in Pakistan on the peanut and peanut products available locally or on the effect of microwave processing on detoxification of aflatoxins.

MATERIALS AND METHODS

Peanut and peanut products were obtained from different vendors and shops. Consumers of these products are generally low income and middle class groups. The low-income group also consumes the low quality of peanuts in the homemade products. Chemicals and solvents used in the analysis were of analytical grades. Aflatoxin standards were purchased from Sigma Chemical Company, St. Louis, MO, and USA. Precoated TLC plates of Silica gel 60 (layer thickness 0.25mm, 20cm x 20cm) on glass or aluminum, without fluorescent indicator were purchased from E. Merck, Dramstadt, Germany. The microwave oven used was of Frigidaire R model RCM5130 with temperature probe. Detoxification procedure was used for the microwave cooking at the heating set approximately up to 92^{o}C with maximum time of 5 minutes. Aflatoxins were determined as described in the Methods of the A.O.A.C. official method 975.36/968.22 [16], except that the defatting of the acetone extract of the sample was performed. TLC plates were observed under long wavelength UV light in an enclosed viewing cabinet and samples were quantified by visual comparison with standards. All positive findings of aflatoxin B_1, naturally present were confirmed by spraying the TLC plates with 50% sulphuric acid and making the derivative with trifluoro-acetic acid. The method is ISO-17025 accredited dually by national and international accredited bodies.

RESULTS

Different samples of peanut and peanut products, like brittle toffees and slabs, peanut butter and of salted-roasted peanuts were analysed and the results are shown in Table **1**. All the contaminated samples were found to contain high levels of both aflatoxin B_1 (from 5.0 to 183.2µg/kg) and aflatoxin B_2 (from 7 to 46.7µg/kg). The samples were exposed to microwave heating which resulted in 55% reduction in the levels of aflatoxin B_1 whereas aflatoxin B_2 was destroyed or reduced to not found within detectable limits *i.e.* ≤1.0 µg/kg (Table **1**).

DISCUSSION

The contaminated peanut and peanut products when exposed to microwave heating resulted in effective degradation of both aflatoxin B_1 and aflatoxin B_2. (Table **1**).

Aflatoxins are quite stable up to their melting point of around 268^{o}C [17]. Dry heating has not been particularly effective while heating moist meal or autoclaving groundnuts has been found to reduce aflatoxin content. Not all aflatoxins react equally to heating, for example aflatoxin B_1 is heat stable, but aflatoxin G_1 can be destroyed by heat [8, 9]. Our results are in line with these finding but microwave heating prove much better in reducing the aflatoxin levels than the ordinary dry heating.

Table 1: Effects of Microwave Processing on Aflatoxin Contents of Peanut and Peanut Products

S. No	Samples	Aflatoxin Content (µg/kg (ppb))					
		Before microwave treatment		After microwave treatment		B_1 % Detoxified	B_2 % Detoxified
		B_1	B_2	B_1	B_2		
1	Salted peanut	5.0	N.D	N.D	N.D	100	------
2	Peanut Toffee Grade I	183.2 ± 15.4	46.7 ± 10.1	100.3 ± 5.0	N.D	54.7	100
3	Peanut Toffee Grade II	175 ± 16.5	7.0 ± 1.4	89.4 ± 3.0	N.D	51.1	100
4	Peanut slab Grade I	116.6 ± 15.0	8.0 ± 1.6	73.8 ± 6.0	N.D	63.3	100
5	Peanut slab Grade II	5.0	N.D	N.D	N.D	100	------
6	Peanut slab Sugar/Bleached	5.0	N.D	N.D	N.D	100	------
7	Peanut Butter	18.7 ± 2.2	N.D	N.D	N.D	100	------
8	Peanut Butter	23.3 ± 4.7	N.D	13.4 ± 3.2	N.D	57.5	100

ND = Not detected within limits.

Mean of 3 replicates ± standard error

Prolonged heating may adversely affect the quality of the protein or availability of lysine [8]. The exposure time in microwave heating is much less and therefore may not adversely affect the quality of the protein or availability of lysine.

Roasting has been reported in some cases to reduce aflatoxin levels, but in no case the total destruction has been achieved [8, 12]. While our study shows that microwave treatment resulted in 55% destruction of aflatoxin B_1 while aflatoxin B_2 was reduced to not found within detectable limits.

It has been reported that dry oven heating resulted in the maximum reduction of aflatoxin B_1 80.2% and aflatoxin B_2 69.7% of naturally contaminated samples of peanuts with initial aflatoxin concentration of 174 and 25 µg/kg at 150 °C and 130 °C, respectively [18]. Though in our study the initial concentration of aflatoxins B_1 and B_2 were 183.2 and 46.7 µg/kg respectively, the microwave heating reduced the aflatoxin B_1 55% while aflatoxin B_2 was reduced to not found within detectable limits.

The present findings clearly indicate that the aflatoxin B_1 was much above the European Union (EU) permissible limits of 2µg/kg in peanut and peanut products which were purchased from local market. This indicates the fact that the use of low quality materials and sub-standard storage conditions before processing, facilitated the growth of the fungal genera *Aspergilli*, thereby, increasing the aflatoxin levels. Hence, peanut products that are commonly available in the local markets may prove to be hazardous for human health, unless proper measures to reduce or eliminate these toxins are adopted. The findings of this research study indicate that microwave treatment would help to some extent to reduce the aflatoxins content of food and food products in a limited time i.e about 5 min.

Substantial work has been put forth on methodologies for the detection and quantification of aflatoxins, however, sampling is of prime importance as to have a representative sample of the total lot under assessment, secondarily sample handling and finally the analysis. It is very unfortunate that sampling, sample handling and analysis is not standardized on part of growers, farmers, producers and ultimately the consumers whom are at great risk. Care must be taken in elucidation and evaluation of results.

The peanuts and peanut products should be stored in a dry and cool environment as the ideal temperature and humidity for fungal growth is line between 86-96°F (30-36°C) and the high humidity.

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