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Original Article

Measurement and assessment of aflatoxin B1 and its producing molds in Iranian sausages and burgers

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Abstract

Introduction: Aflatoxin B1 (AFB1) is one of the most well-known hepatocarcinogens in humans. Contamination of raw materials, used in the production of sausages and burgers, with aflatoxin producing molds can lead to increased level of aflatoxin in the final products and can impose hazards to human health. Unfortunately, aflatoxin is resistant to heating and freezing processes, etc. and can remain in these products untile consumption.

Methods: During a six-month period, 45 sausage and 53 burger samples from valid brands across the country were randomly purchased from the stores. The samples were analyzed for AFB1 by ELISA technique. Meanwhile, the number of molds was calculated and aflatoxin producing molds were identified by direct and slide culture methods.

Results: The findings showed that 2 susage samples (4.9%) and 3 burger samples (6.3%) were contaminated with >1 ng/g aflatoxin. Moreover, 4 burger samples (8.9%) contaminated with mold included aspergillus flavus, aspergillus niger, mucor, and penicillium while, none of the susage samples showed mold contamination.

Conclusion: The Iranian meat products had a relative aflatoxin B1 contamination during the study period, but the contamination rate was low and in allowable range. Standard hygienic preparation and packaging of meat products molds is recommended to reduce fungal contamination, especially aflatoxin-producing molds.

Introduction

Aflatoxins are chemically a large group of mycotoxins with unfavorable carcinogenic, teratogenic and mutagenic effects on humans and animals (1). Aflatoxins are produced by species like aspergillus flavus, aspergillus parasiticus, and aspergillus nomius as well as different species of penicillium, rhizopus, mucor and streptomyce (2). Among the known aflatoxins, AFB1 has more detrimental toxic effects. This toxin is found and can be measured in different types of grains, oild seeds, spices, condiments and the meat of animals that have used contaminated diets (3).

To produce sausage, Kielbasa and burger, in addition to meat which is mainly calf, vegetable oil and depending on the item, soy, gluten, spices, pistachio, beans, mushrooms, and other vegetables are used. Initial contamination of any of these products with aflatoxin can be transmited to the end product. Since pasteurization temperature is used for the production of sausage and other heated products and the heating process is not carried out for burger production except in the cooking phase in which aflatoxins remain unchanged, the health of the consumers will be at risk if a high level of toxin exists in products.

Because of proper conditions of soybean meal, different flours, spices, gluten and other spices for growth of various molds, it is necessary to consider the favorable quality of these items because the presence of aflatoxin producing molds in these products greatly increases the possibility of toxin production in proper temperature and humidity. So far, different methods have been used for measurement of aflatoxin in food products. They include such methods as TLC, HPLC, GC, MS and ELISA, among which ELISA is more acceptable and has a higher accuracy (4).

Measurement of aflatoxin and separation and identification of its producing molds in various food products have attracted many researchers in many countries. In this regard, several studies have been conducted on beef burger, hotdog, sausage, luncheon, ground meat, canned meat and 130 samples of spices used in food industry (5), salted and flavored Egyptian meat (6) and fresh and sun-dried meat (7). Other studies have evaluated the luncheon meats, smoked meats, street foods, different foods for human consumption and even foods for pets in various countries (8-12). In Iran, numerous studies have been carried out to determine the contamination of grains, fodder and animal feed (13,

14). However, there are much data concerning the contamination of meat products like burger and sausage. Nevertheless, all the above-mentioned information indicates that the meat samples and meat products can be contaminated with different aflatoxin producing molds and high levels of aflatoxin. Thus, the current study was aimed to determine and identify the aflatoxin producing molds and to measure aflatoxin B1 level in different sausages and burgers produced by Iranian meat industries.

Materials and methods

Sample preparation

Over six months of visiting the retail stores of Ahvaz, Iran, a total of 45 samples of fresh sausages, including cocktail, hotdog, Bulgarian and Japanese sausages and 53 frozen burger samples with different percentages of meat from valid brands around the country and different production dates were purchased and transferred to the food hygiene laboratory under cold condition, on which the required experiments were performed.

Counting and identification of molds

Under sterile conditions, 25 gr burger or sausage was completely chopped to which 225 mL sterile physiologic serum was added and placed in a stomaker. The, sequential dilutions were made according to the conventional routines, and 100 µl was removed from each dilution by sampler and spread on Sabouraud dextrose agar plate (Merck, Germany). After being dried, the plates were kept in incubator at 25 ºC for 3-5 days and were controlled daily in terms of fungal contamination. The mold and yeast colonies grown in the medium were counted and the number of colonies per 1 gr product (cfu/g) was separately calculated (15). To identify the molds according to conventional methods, in addition to analyzing the appearance, surface color, color of back of colony as well as type (powdery, snowy, filamentary, felty), they were investigated by direct slide and slide culture methods.

To prepare direct slide, some of the given mold was put on the slide containing one drop lactophenol cotton blue. It was covered with adhesive tape and analyzed under microscope. For slide culture preparation, 1×1 squares were prepared from Sabouraud dextrose agar medium and placed in a sterile glass plate containing filter paper, U-shaped tube and slide. Some mold was picked and cultured in the middle of each side of agar square. A total of 10 mL distilled water was added to the plate to prevent agar from drying. The plate was incubated at 22-25 ºC for 2-3 days. The grown molds were analyzed under microscope and identified according to the form of conidia and other characteristic signs.

Measurement of toxin level

For extraction, according to the kit manufacturer's instructions (EuroProxima, Netehrlands), 3 gr of the sample was weighed and 9 mL 80% methanol was added to it and vortexed at laboratory temperature for 10 minutes. After the samples were centrifuged at 2000 g/min for 10 minutes, 50 µl of supernatant was removed and mixed with 150 µl dilution buffer to obtain a solution with 20% methanol. To perform ELISA, 50 µl of this solution was picked and placed in the kit well. The kit was placed in spectrophotometer for ELISA (Biotek Co, USA) and the OD of all wells was read at the wavelength of 450 Nm. The aflatoxin level (µg/l) of the samples was calculated by the drawn calibration curve.

Statistical analysis

For comparison of the contamination level of burgers and sausages, the data were fed into SPSS-16 software and analyzed by descriptive and inferential statistics (Mann-Whitney).

Results

Mold and yeast contamination

The findings indicated that from 45 sausage samples, 14 (31.11%) samples were contaminated with yeast while none of them had mold contamination (Table 1). In addition, the results showed that from 53 burger samples, 23 (43.4%) samples had yeast contamination and 4 (8.9%) were contaminated with aflatoxin producing molds. The type of burger, contamination level and type of detected mold are presented in Table 2.

Aflatoxin contamination

The findings showed that all sausage and burger samples except two burger samples were contaminated with aflatoxin B1. Table 3 presents the number of samples, aflatoxin B1 level, contamination level and standard deviation of sausage and burger samples.

In addition to what has been presented in Table 4, the maximum level of contamination in a cocktail sausage was measured to be 3.2 ng/g.

Table 1. Number of samples, yeast and mold contamination and mean contamination in sausage and burger

Sample type	Sample count	Yeast contamination (%)	Mold contamination (%)	Mean mold (cfu/g)
Burger	53	23 (43.4)	4 (7.6)	$3*10^2$
Sausage	45	14(31.11)	0 (0)	-
Total	98	37(37.75)	4 (4.1)	

Table 2. Number and type of molds detected in different types of burgers

No	Sample type	Contamination level (cfu/g)	Type of detected molds
1	Ordinary, 30% meat (Co 1)	$3*10^2$	*Aspergillus flavus, Mucor*
2	Special, 60% meat (Co 1)	$2*10^2$	*Aspergillus flavus, Penicillium*
3	Ordinary, 30% meat (Co 2)	$3*10^2$	*Aspergillus flavus, Aspergillus niger*
4	Special, 60% meat (Co 2)	$3*10^2$	*Aspergillus flavus, Aspergillus niger, Mucor*

Table 3. Number of samples, aflatoxin B1 level, mean contamination and standard deviation in sausage and burger samples

Sample type	Total number	>1 ng/g			0.5-1 ng/g			<0.5 ng/g		
		No	Mean	STDV*	No	Mean	STDV	No	Mean	STDV
Sausage	41	2	2.32	1.244	34	0.753	0.084	5	0.448	0.044
Burger	48	3	1.55	0.092	26	0.794	0.124	19	0.421	0.153
Total	89	5			60			24		

*Standard deviation

Table 4. Type, characteristics and contamination level of sausage and burger samples with >1 ng/g contamination

No	Sample type	Sample properties	Contamination level (ng/g)
1	Burger	Special, 60% meat (Co 1)	1.6
2	Burger	Ordinary, 30% meant (Co 1)	1.6
3	Burger	Premium, 90% meat (Co 2)	1.44
4	Sausage	Cocktail, 40% meat (Co 3)	3.2
5	Sausage	Hotdog, 60% meat (Co 4)	1.44

The results of statistical analyses revealed that the mean and standard error of aflatoxin in sausage were 0.79 ng/g and 0.066, respectively while, these values were reported to be 0.69 ng/g and 0.046 in burger. The findings of Mann-Whitney test showed no statistically significant difference between burger and sausage ($p>0.05$).

Discussion

The results of this study showed that both sausage as a heated product and burger as a raw product with no preservatives have yeast contamination, indicating that either the production heat did not completely destroy the yeast in sausage or a secondary contamination occurred. Based on these results, the studied sausages lacked mold contamination, which could be due to thermal operation or the effect of the preservatives in sausage. The results obtained for the burgers indicated a rather low contamination (7.6%) of this product with different molds.

The results of this study can be compared to those of Fazlara et al. in 2006. Over a similar investigation on the ordinary and premium burgers presented in Ahvaz city, they reported a high percentage of yeast and mold contamination. In this study which was conducted on 80 raw burgers (40 ordinary and 40 premium burgers) in summer and winter in Ahvaz city, it was found that 100% of ordinary burgers and 90% of premium burgers in winter and 95% of ordinary burgers and 50% of premium burgers in summer were contaminated with mold and yeast above the Iranian national standard. Also, a significant difference was reported between summer and winter as well as between different regions of Ahvaz city (16). The significant difference in mold and yeast contamination in the burgers presented in Ahvaz city over a ten-year period is highly remarkable. This can be due to strict observation of health regulations or may be indicative of illegal use of preservatives in burgers. Since no heating is applied in the production of this product and it legally lacks preservatives, the relative contamination of this product with mold is noteworthy and needs to be analyzed more cautiously.

It should be noted that in the present study *Aspergillus flavus*, *Aspergillus niger*, mucor and penicillium, which are the most important aflatoxin producing molds, were isolated from the samples, and their presence have to be considered important. Preservation of food products in favorable temperature and humidity can cause the growth of aflatoxin producing molds and risk the health of consumers. Unfortunately, the produced aflatoxin is completely resistant to the conventional heating and is not destroyed. Determining the standard level or maximum tolerance of toxins in different foodstuff is one of the methods used by the monitoring systems to ensure the safety of food products. According to Iranian national standard, the maximum tolerance limit of aflatoxin B1 in food products is 5 ng/g (17), so regular monitoring of food products and comparing them with standard limit can positively affect the development of public health in the community.

Contamination with aflatoxin and its producing molds in the food products has been evaluated around the world out of which many reports have been published. For example, a study was conducted on 215 fresh and processed meat samples such as beef burger, hotdog, sausage, luncheon, ground meat, and canned meat as well as 130 spices used in the Egyptian food industry. The results showed that 5 burger samples, 4 black pepper samples, and 4 white pepper samples had AFB1 contamination (8-35 µg/kg). In addition, a number of other kebab, hotdog, sausage and luncheon samples were contaminated with various levels of AFB1 and AFB2. In the above analysis, 24 cases of aspergilus flavus and 16 samples of aspergilus parasiticus were isolated from the samples (4). Refai et al. investigated aflatoxin B1 level and its producing molds in the salted and flavored Egyptian meat (Basturma) samples. Aspergilus, penicillium, mucor, rhizopus, fusarium and cladosporium were the most common molds separated from Basturma and its components like spices. The total aflatoxin level varied from 2.8 to 47 µg/kg (5). In another study carried out in Nigeria, 80 fresh and sun-dried meat samples collected from five provinces were studied in terms of aflatoxin and mold. The maximum level of contamination was observed in the sun-dried meats. In this study, 18 mold samples, including aspergilus, penicillium, alternaria, cladosporium, fusarium, neospora, rhizopus and yeast were identified, most of which were of aspergilus type. In addition, aflatoxin B1, B2, G1 and G2 were observed in all samples (7). Street snacks containing corn and almond in Nigeria were also investigated in 2012. Aspergilus

flavus was isolated from 36% of samples. Other studies have shown that only 68% of snack samples contain aflatoxin. The maximum level of aflatoxin in the samples was measured to be 0.5 µg/kg (9). In a study carried out in the city of San Luis, Argentina, 515 meat samples, including 315 fresh sausage samples, 100 burger samples and 100 ground meat samples were studied microbiologically. All samples were mold and yeast positive with 10^3-10^5 cfu/g contamination (18).

This has also been considered by the Iranian researchers. For instance, Mohsenzadeh and Khezri (19) analyzed 120 frozen burger samples in Mashhad and reported mold and yeast contamination was above the Iranian standard level (10^3 cfu/g) in 5.8% of samples, which is relatively in line with the results of the present study. Further, Soltani evaluated 19 types of spices in Gooshtiran Company and reported $>10^2$ cfu/g mold contamination in 76.78% of samples. The isolated strains included aspergillus, penicillium, mucor and trichoderma (20). In a study conducted on the microbial contamination of port sausage presented in the delis of Shahr-e-Kord, 70 (47.17%) samples were shown to have mold contamination (21).

All the above studies show that different foodstuffs, including meat products, foods prepared with poor materials and spices used in foods can have relative contamination with different types of pathogenic aflatoxin and its producing molds. These pathogens under appropriate conditions can endanger the health of consumers by producing toxin. Considering the high consumption of meat products in the country, the concerned organizations are required to pay sufficient attention to the production and regular monitoring of these products in order to maintain the public health. Since the molds are able to grow even in refrigerator temperature and storage of foodstuff in refrigerator, including domestic and industrial refrigerators does not prevent their activity (22), it is necessary to regularly pay special heed to the production processes of meat products in factories. Furthermore, use of good quality raw materials, including meat, fat, spice, etc., accuracy in heating process, proper packaging, and controlling the temperature and humidity of refrigerators can be helpful in this regard.

Conclusion

Mold contamination and aflatoxin B1 level in the study samples of this experiment were found to be low and at a standard range, but the presence of mold shows that special care should be given to the selection of raw materials, production hygiene, and preservation of food products in refrigerators. Given the limited number of samples in this study as well as high production and consumption rate of these products in Iran, a more comprehensive analysis is suggested to be carried out on a larger number of samples in different seasons. Moreover, it is recommended to analyze the possibility of illegal addition of preservatives to these products.

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