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Detection of Aflatoxins in Peanut Oils Marketed in Peshawar, Pakistan Using Thin Layer Chromatography

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HIGHLIGHTS

- Prevalence rates of aflatoxin B_1, B_2, G_1, and G_2 in peanut oils were 70, 51.7, 3.3, and 0%, respectively.
- Mean of total aflatoxins was 8.59 µg/kg ranged from 0.12 to 55 µg/kg.
- There was significant difference between aflatoxin levels in the samples from different three areas of Peshawar.
- Totally, 5% of the samples were contaminated with aflatoxin B_1 above permissible limits according to national regulation.

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Acronyms and abbreviations
AF=Aflatoxin
TLC=Thin Layer Chromatography

ABSTRACT

Background: Aflatoxins (AFs) are natural toxins produced by fungus belonging to genus *Aspergillus*. These toxins are the secondary metabolites, which may cause teratogenic, mutagenic, and carcinogenic effects due to contamination of food. Peanut is an economically important crop, grown in many parts of the world. The main aim of this survey was to detect AFs in peanut oils marketed in Peshawar, Pakistan.

Methods: During September 2020 to February 2021, a total of 60 peanut oil samples were obtained from retail stores and markets; 20-each from three different areas of Peshawar (University, City, and Cantt), Pakistan. AFB_1, AFB_2, AFG_1, and AFG_2 were determined using Thin Layer Chromatography. Data analysis was done using SPSS 21.0.

Results: Prevalence rates of AFB_1, AFB_2, AFG_1, and AFG_2 in peanut oils were 70, 51.7, 3.3, and 0%, respectively. The mean of total AFs was 8.59 µg/kg ranged from 0.12 to 55 µg/kg. Totally, 5% (3 out of 60) of the samples were found contaminated with AFB_1 above the permissible limits (20 µg/kg) according to national regulation. There was significant difference (p<0.05) between AF levels in the samples from different three areas of Peshawar.

Conclusion: Although, the majority of samples of peanut oils in Peshawar (Pakistan) were safe for consumption, monitoring of AFs must be carried out on a regular basis in the case of peanut oil consumed in this region. This study suggested that farmers, food processors, and local processors should be aware of acceptable hygiene practices for the cultivation, protection, transportation, processing, and handling of peanut oil.

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Introduction

Aflatoxins (AFs) are natural toxins produced by fungus belonging to genus *Aspergillus*, mainly *A. flavus* and *A. parasiticus*. These toxins are the secondary metabolites, which may cause teratogenic, mutagenic, and carcinogenic effects due to contamination of food. Most AFs (including AFB_1, AFB_2, AFG_1, and AFG_2) are mainly

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found in plant based foods such as cereals, grains, nuts, tea, cocoa, spices, edible oil, etc. (Gnonlonfin et al., 2013; Gong et al., 2016; Idris et al., 2010). There are some chemical and physical methods that may be used for degradation or reduction of AF in plant edible oils. UV and gamma irradiation are of the most known physical methods for this purpose. Chemical agents like alkaline solutions, ozone, and electrolyzed water are also proposed. However, efficacy of biological methods has not been proved yet for decontamination of AF in the edible oils (Javanmardi et al., 2020; Pankaj et al., 2018).

AF contaminations in foods are one of the most important food safety concerns in the world. Peanut (*Arachis hypogaea*) is an economically important crop, grown in many parts of the world. Peanut is used in the processing of oil, human food, in agricultural, and animal feed sectors. AF may be produced subsequently in the kernels due to fungal invasion especially by aflatoxigenic fungi (Bankole et al., 2005; Mutegi et al., 2009). Production and trade of peanut is faced various challenges due to mycotoxin producing fungus. Most food processors, farmers, and food handlers lack knowledge on the effects of pathogenic mold production, in particular the prevalence of AFs. Many developing and developed countries have set maximum permissible limits for AFs in different food commodities (Chang et al., 2013; Mutegi et al., 2009; Rushing and Selim, 2019; Soler et al., 2010).

Knowledge in this field of research is minimal in Peshawar region of Pakistan. Awareness and data regarding health risk associated with utilization of AF contaminated peanut and peanut oils are not reported in this region. This research provides baseline information on frequency and contamination levels of AF in selected peanut product in this area. So, the main aim of this survey was to detect AFs in peanut oils marketed in Peshawar, Pakistan using Thin Layer Chromatography (TLC).

Materials and methods

Samples collection

During September 2020 to February 2021, 60 peanut oil samples were obtained from retail stores and markets; 20-each from three different areas of Peshawar (University, City, and Cantt), Pakistan. The samples were brought to Mycotoxins Laboratory, Food Technology Centre, PCSIR Laboratories Complex Peshawar and were stored at 4 °C till analysis.

Chemicals

In the present study, Merck (Darmstadt, Germany), BDH (Poole England) and Sigma Chemicals (ST. Louis, USA) analytical grades chemicals were used. The Biopure (Tecknopark Tullin, Austria) standards of AFB_1 (2.02 µg/ml), AFB_2 (0.500 µg/ml), AFG_1 (2.02 µg/ml), and AFG_2 (0.500 µg/ml) were used which were prepared concentration of 1 µg/ml by diluting in benzene/acetonitrile (98:2; v/v), wrapped with aluminum foil, and stored at 4 °C until the analysis.

Analysis of AFs

In this survey, the AFs levels in the samples were determined using TLC. The test sample was weight 50 g and extracted with 250 ml acetone/water (85:15; v/v) using blender for 3 min. Then, the sample was filtered by the using of whatman filter paper. The filtrate sample (150 ml) was collected; then 170 ml of 0.02 N sodium hydroxide, 30 ml ferric chloride, and 3 mg of basic copper carbonate were added. The solution was properly mixed and again was done filter, then 150 ml of filtrate was transferred to separating funnel. Sulphuric acid (H_2SO_4) 0.03% was added to the filtrate. The sample was two times extracted with 10 ml of chloroform. Also, 100 ml of 0.02 M potassium hydroxide was added to the lower chloroform layer and transferred to another separating funnel. The separating funnel was swirled gently for 30 s and wait for layer separation. Then, the lower chloroform layer was transferred into vials. Next, 8 ml of chloroform layer was heated at 45 ^{o}C until the chloroform evaporates. For TLC, the residue was dissolved into 200 µl benzene/ acetonitrile (98:2 v/v).

One-dimensional TLC on pre coated silica gel plates (Merck, Germany) were performed for the identification and quantification of total AFs. The saturated chamber of chloroform/xylene/acetone (60:30:10; v/v/v) were prepared and the plates were developed inside the saturated chamber. For the comparison of AFs samples and standards, spot of 365 nm ultraviolet light were used. Using 50% H_2SO_4 spray and trifluoroacetic acid (TFA) reaction, the AFs were conformed (Scott, 1984).

Statistical analysis

Data analysis was done using SPSS 21.0 (Window version, IL, USA). The one-way ANOVA procedure was done for significance at 95% confidence level for means distribution of AFs concentration in peanut oils. For the separation of means Duncan's multiple range tests was used.

Results

The levels of AF contamination contained in peanut oils from the three main areas of the Peshawar region are shown in Table 1. Prevalence rates of AFB_1, AFB_2, AFG_1, and AFG_2 were 70% (42 out of 60), 51.7% (31 out

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of 60), 3.3% (2 out of 60), and 0%, respectively (Table 1). The mean of total AFs was 8.59 µg/kg ranged from 0.12 to 55 µg/kg.

Among all the samples, 5% (3 out of 60) were found contaminated with AFB_1 above the permissible limits (20 µg/kg) according to National Agency for Food and Drug Administration and Control, while 41.7% samples (25 out of 60) had AF concentrations above the permissible limit for AFB_1 (2 µg/kg) and 35% samples (21 out of 60) for total AFs (4 µg/kg) according to European Union standards (Table 2). There was significant difference (p<0.05) between AF levels in the samples from different three areas of Peshawar (University, City, and Cantt) in Pakistan.

Table 1: Contamination levels of aflatoxins in peanut oil samples collected from three main areas of Peshawar, Pakistan

Aflatoxin	Areas of Peshawar	Frequency of contamination	Range (µg/kg)	Mean±SD (µg/kg)
Aflatoxin B_1				
	University	13/20	0.30-18	5.58±5.27 [b]
	City	15/20	0.12-22	5.34±6.40 [b]
	Cantt	14/20	0.30-25	7.69±7.96 [a]
Aflatoxin B_2				
	University	10/20	0.6-10	2.64±2.38 [b]
	City	9/20	0.2-11	2.88±3.22 [b]
	Cantt	12/20	0.2-22	4.69±5.60 [a]
Aflatoxin G_1				
	University	0/20	ND	ND
	City	0/20	ND	ND
	Cantt	2/20	2.4-7.4	4.90±1.65
Aflatoxin G_2				
	University	0/20	ND	ND
	City	0/20	ND	ND
	Cantt	0/20	ND	ND
Total aflatoxins				
	University	13/20	0.30-27	7.02±7.37 [b]
	City	15/20	0.12-29	7.03±9.26 [b]
	Cantt	14/20	0.30-55	11.73±10.72 [a]

ND: Not Detected
-Means followed by the same letter do not differ significantly according to Duncan Multiple Range Test (DMRT) at 5% level of significance.

Table 2: Number (%) of peanut oil samples from Peshawar, Pakistan exceed maximum limit for aflatoxin B_1 and total aflatoxin

Sampling areas	Aflatoxin B_1 (µg/kg)				Total aflatoxin (µg/kg)		
	Range	Mean	>2*	>20**	Range	Mean	>4***
University	0.30-18	5.58 [b]	09/20 (45%)	0/20 (0%)	0.30-27	7.02 [b]	9/20 (45%)
City	0.12-22	5.34 [b]	06/20 (30%)	1/20 (5%)	0.12-29	7.03 [b]	4/20 (20%)
Cantt	0.30-25	7.69 [a]	10/20 (50%)	2/20 (10%)	0.30-55	11.73 [a]	8/20 (40%)
Total	0.12-25	6.20	25/60 (41.7%)	3/60 (5%)	0.12-55	8.59	21/60 (35%)

* Number (%) of samples contaminated with >2 µg/kg level of aflatoxin B_1 according to European Union regulation.
** Number (%) of samples contaminated with >20 µg/kg level of aflatoxin B_1 according to National Agency for Food and Drug Administration and Control.
*** Number (%) of samples contaminated with >4 µg/kg level of total aflatoxin according to EU regulation.
-Means with different superscript letter in a column are significantly different at 5% level of significance.

Discussion

Depending on economic situation, the legal AFs limits in foods may differ from one country to another. The maximum permissible amount of 2 µg/kg for AFB_1 (Commission Regulation, 2001) has been governed by the Scientific Commission of the European Community.

In this survey, we found AFB_1, AFB_2, AFG_1, and AFG_2

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in the peanut oil samples of Peshawar region with rates of 70, 51.7, 3.3, and 0%, respectively. Similar with our results, Qi et al. (2019) stated that among 427 peanut oil samples from western Guangdong, China, AFB_1 was found in 47 samples (22.5%) ranged from 15.4 to 49.9 µg/kg. Ndiaye et al. (1999) showed that 80% of peanut oil produced in the Kaolack and Diourbel areas of Senegal were contaminated to AF with a mean total level of 40 µg/kg. Elzupir et al. (2010) found AF in 98.8% of vegetable oil samples in Khartoum province, Sudan. These researches stated that total AFs range in vegetable oil samples was 0.43-339.9 µg/kg with a mean of 57.5l µg/kg which was too higher than what we found in the current study (range: 0.12 to 55 µg/kg; mean: 8.59 µg/kg).

Recently, findings of a new research from China have revealed considerable AF-contamination in peanuts consumed in this country. The most AF exposure levels were reported from South and East of China which may endanger public health (Qin et al., 2021). According the mentioned report, the estimated dietary exposure to AF from the total of peanuts and peanut oil for Chinese people ranged from 1.776 to 1.940 ng/kg bw/day. The 2-6 years Chinese children had the most AF exposure, comparing to the other age groups in this country. Based on another survey in Haiti, AFs levels in raw peanuts, peanut butters, and maize samples were 14, 97, and 30%, respectively. The highest AF level was found in peanut butters with maximum level of 2720 µg/kg (Schwartzbord and Brown, 2015). According to a study in Punjab (Pakistan), occurrences of AFs in samples of raw peanut with shell and raw peanut without shell were 59 and 61%, respectively. Also, mean levels of AFs in raw peanut shell and raw peanut without shell were 6.4 and 9.6 µg/kg, respectively (Iqbal et al., 2013). However, less information on the occurrence or levels of AFs in peanut oil consumed in Pakistan is available; thus, the extent of the health risk from the ingestion of contaminated oils with AFs remains unclear.

Schwartzbord and Brown (2015) proved that AF level in peanut oil is approximately 5% of the original AF-contaminated peanuts. They concluded that pressure extraction process could be considered as a low-cost method for reduction of AFs in peanut oil obtained from AF-contaminated peanuts. However, considering the results of the present study, there are still some concerns due to AF levels in the final peanut oil products distributed in Pakistan. So, it is essential to apply some degradation practices for reduction of this toxin in the final peanut oil products. For example, some degradation methods such as photodegradation of AF under UV irradiation have been proposed for reduction of this mycotoxin in the peanut oils. Thus, it is stated that UV irradiation reactors could be used in a large scale in oil industries for reduction of AF in peanut oils (Diao et al., 2015; Liu et al., 2011; Mao et al., 2016).

Conclusion

Although the majority of samples of peanut oils in Peshawar (Pakistan) were safe for consumption, monitoring of AFs must be carried out on a regular basis in the case of peanut oil consumed in this region. Continuous surveillance measures must be done for reduction of AFs risks in the local consumers. This study suggested that farmers, food processors, and local processors should be aware of acceptable hygiene practices for the cultivation, protection, transportation, processing, and handling of peanut oil.

Author contributions

A.H. and Z.R. designed the study, did the experimental work, and analyzed the data; M.K. wrote the manuscript and analyzed the data. All authors revised and approved the final manuscript.

Conflicts of interest

There is no conflict of interest in the study.

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