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Incidence of Aflatoxin in Ready to Eat Nuts from Local Food Markets in Mangaluru, India

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Abstract

Background and Objective Aflatoxins are a group of naturally occurring mycotoxin which are toxic secondary metabolites produced by certain filamentous fungi like *Aspergillus flavus* and *Aspergillus parasiticus*. The main objective of this study was to screen the occurrence of aflatoxin in ready to eat nuts available locally and analyzing for its nutritive value and to evaluate the efficiency of conventional (thin-layer chromatography [TLC]) and sensitive kit-based (enzyme-linked immunosorbent assay [ELISA]) method by detection of the aflatoxin in the sample.

Methods A total of 50 samples including peanuts (10), cashew nuts (10), almonds (10), pistachio (10), and walnuts (10) were collected from different stores in Mangalore city. Each sample was divided into three fractions, as for microbiological analysis, proximate analysis, and detection of aflatoxin by following standard method (AOAC2000).

Results The present study evidenced the contamination of aflatoxin in all of the five types of ready-to-eat nuts examined and the concentration was within the acceptable limits. But, among the samples analyzed, G10 (groundnut) showed a maximum concentration of 16 µg/L aflatoxin detected by ELISA method. It was also observed that the proximate analysis mainly moisture content did not affect aflatoxin accumulation.

Conclusion Our study shows that aflatoxin contamination of food products has become a serious threat. Although several methods for detection and quantification of toxins have been developed, due to their low concentration of toxicity in food commodities, an analytical method for detection and quantification of aflatoxin have to be specific, sensitive, and simple to carry out and among TLC and ELISA, ELISA came out as a suitable for rapid and sensitive detection.

Keywords
- mycotoxin
- aflatoxin
- proximate analysis
- fungus

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Introduction

Aflatoxin-contaminated agricultural commodities always remain a prime concern which shows a carcinogenic property globally, especially in warmer and humid climatic regions.[1] The term aflatoxin is derived from the name of the fungi *Aspergillus flavus* as it was first isolated from this species.[2] Reference of aflatoxin B1 and B2 are commonly made as to the most common toxins which contaminate mainly cereals, and aflatoxin M1 and M2 are the hydroxylated metabolites of B1 and B2, respectively, known as milk toxins.[3]

The agricultural commodities contaminated by aflatoxin include maize,[4] seeds of apricot, walnut, sunflower seeds, kernel, sesame seed, almond, peanuts, pistachio, hazelnuts, cashew nuts,[5] pine nuts, peanuts,[6] brazil nuts,[7] dried plums, dates, raisins, watermelon seeds, spices, and corn.[2]

The fungi producing aflatoxin depends on various substrates and ecological conditions such as temperature, pH, light, moisture, relative humidity, water, storage condition, preservatives, redox potential and microbial growth. The ideal temperature for their growth ranges from 12 to 42°C and the optimal temperature is 25 to 35°C.[8] Aflatoxin affects the agricultural commodities during pre and post-harvesting. Storing under different climatic condition and storage under adverse temperature also affect the growth of aflatoxin. Along with these parameters, relative humidity also influences the toxin production. Insects and rodent damage, excessive heat, and lack of aeration also cause growth of aflatoxin. Aflatoxin is a carcinogenic, immune-suppressive, mutagenic, and teratogenic toxin.

Therefore, the study mainly focuses on screening the occurrence of aflatoxin in ready-to-eat nuts available locally, and the screened nuts will be analyzed for its nutritive value.

Methodology

Sample Collection

Samples like ready to eat nuts (peanuts [10], cashew nuts [10], almonds [10], pistachio [10], and walnuts [10]) were collected from different stores in Mangalore. The samples were collected randomly from the different local grocery stores once in fortnight for a period of 6 months (October–March). The average annual percentage of humidity encountered in the city is approximately 77%. Both loose (plastic bags) and packed dried fruits were included in this study. The samples were stored in the refrigerator at 4°C until further use.

Isolation of Fungi for Microbiological Analysis

Present study used Sabouraud Dextrose agar (SDA) for the isolation of fungi from nuts. Samples (1 gm) were homogenized with 0.85% saline, serially diluted. A 0.1-mL sample from each dilution was spread on the SDA. The plate was then incubated at 30°C for 5 to 7 days. Obtained fungal isolates were examined for the macroscopic characteristics (colony color, morphology, and size). Isolates were identified to genus level using appropriate manuals.[9]

Proximate Analysis

The proximate composition (moisture, ash, crude fiber, protein, fat, and carbohydrates) of the nuts were performed according to the procedure explained in Association of Official Analytical Chemist (AOAC; 2000).[10]

Detection of Aflatoxin

Enzyme-Linked Immunosorbent Assay and Thin-Layer Chromatography

Approximately 25 g of ready to eat nuts were homogenized with the solvent mixture (methanol:water mixture = [8:2]). Obtained homogenates were filtered and filtrate were used for ELISA and TLC analysis.

Detection of aflatoxin by ELISA method was done by kit based method (RIDASCREEN Aflatoxin total: R4701). Thin-layer chromatography (TLC) was performed as mentioned in the AOAC (2000) method.

Results

Proximate Analysis

The samples were subjected to estimate moisture, ash, fiber, carbohydrate, fat, and protein content. The results obtained were depicted in the ►**Table 1**.

Microbiological Analysis

All the ready-to-eat nuts used in the study showed growth of fungus on SDA. The fungal species belonging to three major genera (*Rhizopous*, *Pencillium*, and *Aspergillus*) were isolated.

The samples like ground nut, pistachio, and cashew showed positive for *Aspergillus spp*.

Table 1 Proximate analysis of ready to eat nuts

Sample		Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	Carbohydrate (%)
Groundnut	Sample	3.12 ± 0.11	2.2 ± 0.14	25.18 ± 0.5	45 ± 0.83	2.9 ± 0.21	22.9 ± 0.30
Almond	Sample	4.8 ± 0.70	2.6 ± 0.22	20.2 ± 0.86	59 ± 0.40	1.5 ± 0.11	11.4 ± 0.31
Walnut	Sample	4.3 ± 0.42	1.7 ± 0.22	15 ± 0.32	64.3 ± 0.12	2.6 ± 0.21	12.1 ± 0.11
Pistachio nut	Sample	5.7 ± 0.64	2.7 ± 0.21	19.5 ± 0.46	53 ± 0.30	2 ± 0.13	17.6 ± 0.20
Cashew nut	Sample	5.5 ± 0.67	2.3 ± 0.31	21 ± 0.51	46.2 ± 0.62	1.3 ± 0.12	23.5 ± 0.22

Note: Results are mean of triplicate analysis.

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Quantitative Estimation of Aflatoxin

The concentration of aflatoxin in the ready-to-eat nuts were checked using a solid phase direct competitive enzyme immunoassay. The present study revealed the presence of aflatoxin in all the five types of ready to eat nuts such as groundnut (0.078–16.1 µg/L), almond (0.19–0.55 µg/L), walnut (0.1–3.4 µg/L), pistachio (0.78–1.69 µg/L), and cashew nut (5.18–10.73 µg/L). However, the concentration was within the acceptable limit. But, among the samples analyzed, groundnut (G10) showed a maximum concentration of 16 µg/L aflatoxin. The detection limit of ELISA kit used in this study is 1.75 µg/kg according to the manufacturer's detail.

The result obtained from the ELISA was compared with the gold-standard method TLC which was widely used and has been used as a standard method by the Association of Official Analytical Chemist (2000). Among 10 samples of groundnut, three samples (G2, G9, and G10) exhibited blue fluorescence, indicating the presence of B1 aflatoxin in the sample. Almond and walnut did not show any fluorescence's under ultraviolet (UV) light. Pistachio and cashew nut showed blue fluorescence that is positive for all the samples analyzed.

Discussion

Aflatoxin is considered to be a carcinogenic substance that is produced by certain types of fungi which are naturally found in the environment. They contaminate the food and cause a serious threat to humankind, as well as livestock products. Consumption of nuts can be considered as a healthy practice provided it is not consumed daily. The present study evidenced the contamination of aflatoxin in all five types of samples. However, the concentration was within the acceptable limit. But, among the samples analyzed, groundnut (G10) showed a maximum concentration of 16 µg/L aflatoxin detected by ELISA method. However, 15 µg/kg is the maximum consumable limit prescribed by the World Health Organization (WHO).[11]

Although aflatoxins in walnut, almond, pistachio, cashew, and groundnut were detected by ELISA, almond and walnut did not show any positive for aflatoxin by TLC method. Most of the studies report that TLC is considered to be the best standard method for detecting aflatoxin. This study reveals that the ELISA is the sensitive method and the sample with lower concentration up to 0.1 µg/L can be detected. Therefore, it can be considered that ELISA is a more sensitive and rapid method to detect aflatoxin and similar conclusions have been drawn by other researchers. Negative result of TLC may be due to preparation/application of samples, plate development, and plate interpretation.[12] Preresult study observed high percentage of moisture in the sample may also be as a result of improper packaging or lack of use of good packaging materials. Nuts are hygroscopic that collect moisture from the surrounding environment until equilibrium is reached.[13] Harvesting methods may also have an impact on the moisture content of the nuts. In this study, the samples were collected from local markets of Mangalore city where the district encounters more rainfall, maximum humidity, and tropical climatic conditions that can pose adverse effects on quality of ready to eat nuts. During sample collection, it was observed improper storage conditions, that is, the retailers stored the nuts in easy-open access containers which were transferred from gunny/plastic bags.

The storage conditions which had to be maintained critically were ill maintained. Also, it was noted that the poor handling of the products can be one of the crucial factors for aflatoxin contamination. The favorable temperature for the growth of fungi ranges from 25 to 32°C.[14] *Aspergillus spp*. can easily grow at optimal conditions of 27 to 33°C, pH range of 5 to 6 and water activity of 0.82 to 0.99. The growth of *Aspergillus Rhizopous*, and *Pencillium* in nuts suggests the improper handling of the samples by the retailer. The occurrence of these fungus in present sample complemented the result of previous researchers[15,16] who reported a similar trend of fungal occurrence.

Conclusion

The aflatoxin contamination of the food products has become a serious threat in tropical and subtropical regions, especially in the developing countries with poor practices. Where the environmental conditions like warm temperatures and humidity favor the growth of fungi. Although several methods for detection and quantification of toxins have been developed, we compared between TLC and ELISA kit where ELISA came out as a suitable kit for rapid and sensitive tests but with ELISA techniques, a positive result needs to be verified by HPLC because no ELISA method has been given AOAC international approval. Furthermore, the food standards should be strictly enforced in the open food market and should have systematic monitoring throughout the food chain.

Conflict of Interest
None declared.

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