Journal of Basic and Environmental Sciences
ISSN
Online:2356-6388
Print:2536-9202

Research Paper Open Access

Occurrences and frequency of fungi isolated from fast foods and spices

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Abstract: Meat products especially beef; luncheon and burgers are some of the most popular meals in many countries in the world including Egypt, which were found to be highly contaminated with fungi, especially *Aspergillus* and *Penicillium* spp. during the manufacturing process leading to public health hazards due to their mycotoxins production. Therefore, the current study focused on the isolation and identification of mycotoxigenic fungi that were associated with the processed food samples. A total of 54 food samples, including luncheon, burgers, sausage, meat spices, basterma, indomie corn flex, spices, crisps, karate (snacks), biscuits, maize, and soybean, were gathered from various locations in the Qalubyia Governorate in Egypt. The collected samples were examined mycologically to evaluate their quality and safety. According to the findings of this study, the luncheon samples under examination had the highest total fungal count of 313 fungal colonies/10g of the tested samples, followed by meat spices (153) and crisps (152) fungal colonies/10g. While, indomie, sausage and soybean samples showed the lowest number of fungal colonies (14, 25, and 25 colonies/10 g, respectively). The recovered fungi were identified morphologically based on macro and microscopic traits and belonged to seven genera. Interestingly, results showed that the genus *Aspergillus* was the most frequently (*A. niger* (87.044 %) and *A. flavus* (59.26 %) followed by *Penicillium* genus which represented (59.26 %). Moreover, the most predominant fungal species were screened for their toxin production. Data cleared that the highest concentrations of aflatoxins and ochratoxin A being (200.03 and 17.26 ng/ mL) were obtained from *A. flavus* and *A. niger,* respectively isolated from basterma. These findings emphasize the risk of fungal contamination exposure to consumers due to the high consumption of fast foods and spices which may be susceptible to fungal infection, leading to mycotoxins contamination if the storage conditions are favorable for the fungal growth.

Keywords: Fast food, spices, *Aspergillus niger*, *Aspergillus flavus, mycotoxins*

1- Introduction

Ready-to-eat (RTE) products, such as luncheon, basterma, and hawawshi are prepared to be eaten without needing cooking and therefore often consumed without additional cooking steps. Post-process handling is a cause of recontamination of RTE meat products especially with food pathogens. Consumers may choose to cook them for a better taste or appearance [1]**.** Some ready-to-eat foods were considered potentially hazardous because such foods can support the growth of microbes. Such food must be kept at certain temperatures and conditions to decrease the growth of any microorganisms that may be found in the food or to prevent toxins formation in the food. Due to the nature of these foods and their methods for preparation involving extensive handling, they were contaminated during storage, distribution facilities, soil, water, air environment, and human activities including the food handlers and vendors [2]. Meat products provide an excellent growth media for a variety of microorganisms[3]**.**

It is believed that there is a big problem with meat products that are contaminated with several types of fungi because it makes decay and disintegration more likely, it has an effect on the meat quality products. The most important side about the fungal spoilage of food is, the formation of mycotoxins especially aflatoxins, which considered the main toxic secondary metabolites of *Aspergillus* spp. such as *A. parasiticus, A. flavus* and *A. nomius* [4,5].

Some fungi especially *Aspergillus* spp. had a bad effect on the human health; it not only causes diseases, but also contaminated the human diets. Some mold species such as *A.flavus* and *A. parasiticus* are toxigenic and produce aflatoxin in foods [6,7]. Since meat products provide a significant amount of (proteins, essential amino acids, fats, minerals, vitamins, and other nutrients) they are more appealing of highly nutritious diets for human's consumptions. They are thought to be the best culture media for the development of many organisms. However, due to their high levels of moisture, large amounts of nitrogenous substances, abundant mineral supply, and presence of some fermentable carbohydrate (glycogen) they were an ideal media for the majority of microbes [8]. Therefore, the growth of some fungal species is dangerous, because they can produce several mycotoxins [9].

Mycotoxin is a problem in the food industry that has an effect on human and animal's health. In storage conditions, fungal bio-deterioration of stored food is a chronic problem especially in tropical hot and humid climates due to the excretion of mycotoxins [10,11], that can be produced by different fungi such as *Aspergillus, Penicillium, Alternaria, Fusarium, Cladosporium, Mucor*, and *Rhizopus* [12]. Some fungi, such as *Aspergillus, Fusarium, Penicillium,* and *Alternaria* have the capacity to create mycotoxins, which are harmful byproducts that can contaminate food under specific conditions [13,14].

Based on estimates, more than five billion individuals ingest contaminated foods every day and are exposed to mycotoxins through unidentified pathways every day [13]. The disease caused by ingestion of mycotoxin called mycotoxicosis [15].

Thus, the aim of this research is to evaluate certain (ready-to-eat) food items available in Qalubyia supermarkets in terms of their mycological quality, and identification of the most frequently fungal species. As

well as, testing the most common fungi for their ability to produce toxins.

2. Material and Methods

2.1. Samples collection

About (54) samples of different meat products and most consumed corn products also different spices were collected from several supermarkets and shop malls in Qalubyia Governorate. The samples were transferred to microbiology lab at faculty of science, Benha University in sterile plastic bags in Ice-Box, according to Chessbrough [16] for mycological analysis.

2.2. Isolation and identification of fungi

Ten grams of each sample was homogenized with 90 mL of 1% peptone water for two minutes in the sterile warring blender. After that, 1mL of the mixture was inoculated separately into petri dish plates containing potato dextrose agar medium. The plates were then incubated for 5-7 days at 28°C. Followed the incubation period, the growing fungal colonies were counted and the frequency of fungi and the proportional percentage of each species within a genus of fungi were determined according to [17]**.** The purified fungal colonies were identified morphologically according to [18, 19]**.** As well as, the most predominant fungal species were deposited at Assuit University Mycological Center (AUMC), Egypt.

2.3. Screening for toxins production

The most frequently fungal species were screened for aflatoxins (AFs) and ochratoxin (OTA) production on yeast extract sucrose (YES) broth medium according to the methodology described by [20]**.** The fungal species were inoculated separately in YES medium and incubated at 28 ^{o}C under static condition for 14 days. After, incubation period AFS and OTA were extracted from the filtrate as reported by [21]**.** Extraction was carried out using 20 mL of chloroform (with 20 mL filtrate), and homogenization for 3 min in a separation funnel. The chloroform phase was filtered through filter paper with sodium sulphate anhydrous and concentrated to dryness using hot plate then determined using HPLC.

3- Results and Discussion

3.1. Isolation and identification of fungi associated with food samples

Based on the cultural and microscopic characteristics The results showed that the isolated fungi from the collected food samples were belonging to seven genera including *Aspergillus*, *Alternaria*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, and *Trichoderma*. The genus *Aspergillus* was the most predominant followed by *Pencillium* genus. Thus, the most frequently fungal species (*A. niger* van Tieghem, *A. flavus var. columnaris*, *Penicillium verrucosum*, and *Aspergillus oryzae* were deposited at Assiut University Mycological Centre (AUMC), Egypt with deposition numbers (AUMC16130, AUMC16129, AUMC16131 and AUMC 16126, respectively) and they were selected for further studies. Photo (1) showed macro- and microscopic traits of the most potent fungi. The results of the present study showed predominance with those reported by [22]**.** Also, the obtained data was in agreement with [23] who found that the genus *Aspergillus* and *Penicillum* were the most common fungi in meat products.

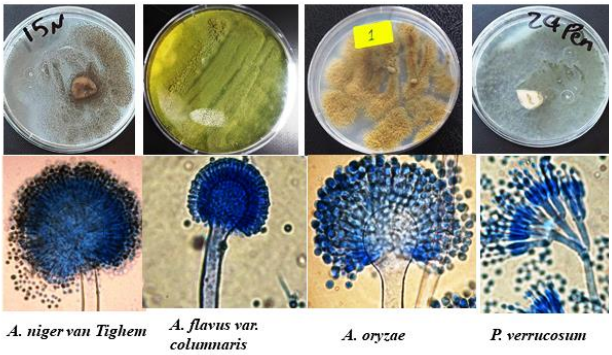

Photo 1. Cultural and microscopic characteristics of the most frequently fungi isolated from food samples.

3.2. Frequency of fungi associated with food samples

Data presented in Table (1) cleared that among the tested samples; *A. niger* van Tighem exhibited the most frequently occurring contaminant and appeared in 47 out of 54 samples with frequency percentage of (87.04 %) followed by *A. flavus var. columnaris* (59.29 %) and *P. verrucosum* (25.93 %). On the other hand, the least fungal frequency (1.85 %) was obtained from *Trichoderma* sp., *Alternaria* sp., and *A. ochraceous*. Our results were in agreement with results obtained by [24].

Additionally, [23] assessed the microbiological quality of meat products as well as spices sold in a number of supermarkets and stores in the Gharbia Governorate, they found that the genus *Aspergillus* and *penicillium* were the most frequently among the samples. The status of meat products is affected by fungi that enter through meat spices, and other components as well as through the processing environment, equipment, and handlers [5]. On the other hand, the environmental conditions in the factories, warehouses, freezers, and stores are favorable for the molds. Although molds can develop inside the products, they do so more frequently on the outside of various kinds of meat and meat products. Significantly to food spoiling, some molds can even produce mycotoxins that can be dangerous to people [25]. As well as, spices have been used in many industries. They frequently have high levels of mold contamination. The most prevalent fungal species that contaminate spices are *Aspergillus* and *Penicillium* spp.[24].

3.3. Occurrence of fungi isolated from the analyzed samples

Data presented in Figure (1) showed that mycological examination of fourteen processed samples, i.e. luncheon, basterma, burger, sausage, meat spices, meat, chicken stock, indomie, corn flex, crisps, karate (snacks), biscuit, corn, and soybean. The greatest total fungal count was found in luncheon samples, giving 313 fungal colonies/10g of the samples under examination, followed by meat spices (153) and crisps (152) fungal colonies/10g. While, the lowest number of fungal colonies was recovered from indomie, sausage and soybean samples which recorded (14, 25 and 25, respectively). In this context, Zohri et al. [25] they found that the fungal population ranged from 164 to 528 colonies / g in 20 beef burger samples. On the other hand, Omorodion and Odu [26] reported that the total fungal count of beef samples ranged from ($6.0x10^4$) CFU/g to ($4.4x10^5$) CFU/g.

The differences in fungal counts may be explained by the geographical location of producing companies, the length of time, these items were held, and the

cleanliness of the personnel handling them. These findings support with [27], who found that the incubation temperature, types of media, and techniques of food analysis are all factors that affect the fungal counts.

Table 1. Fungal frequency associated with examined samples.

Total fungal species	Total No. of isolates	No. of positive samples	Frequency (%)	Relative density (%)
***A. niger* van Tieghem**	790	47	87.04	62.99
A. flavus var. columnaris	395	32	59.26	31.20
A. oryzae	19	5	9.26	1.52
A. ochraceous	1	1	1.85	0.08
P. verrucosum	33	14	25.93	2.63
***Mucar* sp.**	2	2	3.70	0.16
***Rhizopus* sp.**	10	6	11.11	0.79
***Trichoderma* sp.**	4	1	1.85	0.31
***Fusarium* sp.**	5	2	3.70	0.39
A. paraciticus	2	1	1.85	0.15
A. terrus	3	2	3.70	0.23
***Alternaria* sp.**	2	1	1.85	0.15

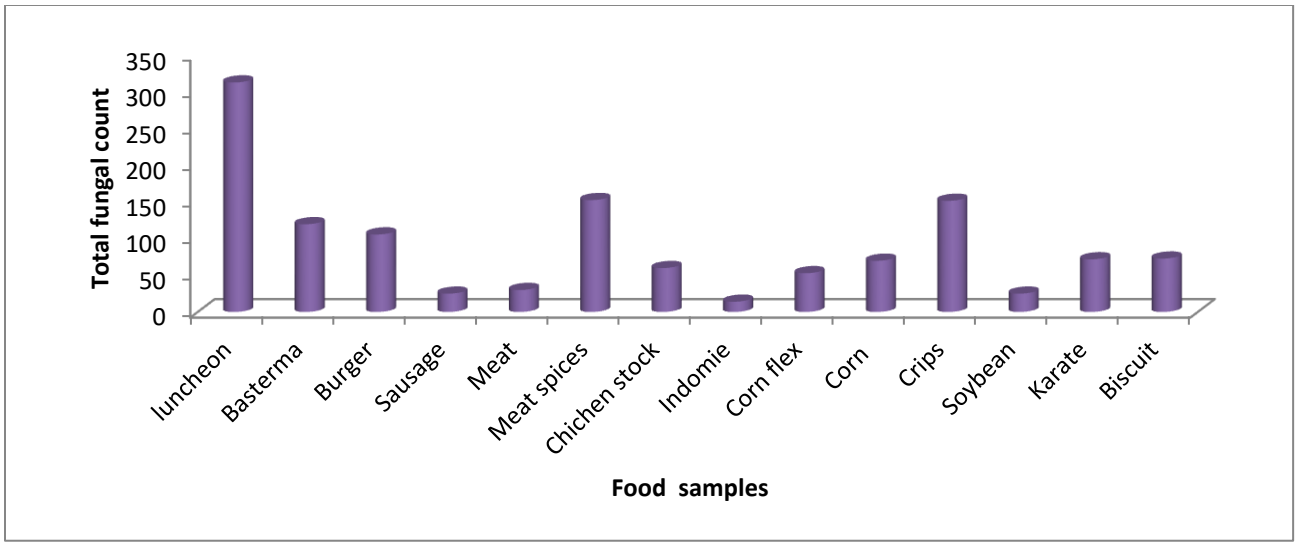

Figure 1. Total fungal count of the examined processed samples.

3.6. Testing for toxins production

Interestingly, the most portent fungal species including *A. flavus var. columnaris, A. oryzae, A. niger* van Tieghem, and *P. verrucosum* are considered the most important fungi for producing carcinogenic aflatoxins and ochratoxins. Data presented in Table (2) and Chart (1 a and b) showed that *A. flavus var. columnaris* isolated from basterma samples exhibited the highest content of the total four types of AFS with 13.27, 91.48, 82.38, and 12. 7 ng/ mL, respectively for AFG1, AFG2, AFB1, and AFB2, respectively, followed by *A. oryzae*.

Table 2. AFs produced by the most common fungi isolated from different food samples.

Type of sample	AFs producer fungi	Concentration of AFs (ng/ mL)				
Basterma	*A. flavus var. columnaris*	AFG1	AFG2	AFB1	AFB2	Total AFs
		13.27	91.48	82.38	12.7	200.03
Luncheon	*A. oryzae*	13.57	1.18	ND	0.837	15.58

*ND: Not detected

On the other hand, the maximum OTA (17.26 ng/ mL) was obtained from *A. niger* van Tieghem that recovered from basterma. While the least amount produced by *P. verrucosum* as shown in Table (3) and Chart 1(c and d). The obtained results clarified that among the examined samples basterma exhibited the highest contamination with the toxigenic fungi and these results were in harmony with those reported by [28] they found that the highest AFs was obtained from bastermna sample. Tirado et al. [29] reported that the AFs are expected to become more prevalent with climate change in countries with temperate climate. Also increased AFs formation was affected by heavy rains during the storage, delayed storage and high moisture contents [30]**.** Additionally, Hamad et al. [31] reported that the highest residues of OTA were obtained from basterma samples, followed by luncheon, kofta, and burger samples. In contrast, the sausage samples yielded the lowest quantity of OTA.

Table 3. OTA resulted from the most potent fungi recovered from food samples.

Type of sample	OTA producer fungi	Total OTA (ng/ mL)
Basterma	*A. niger* van Tieghem	17.26
Corn flex	*P. verrucosum*	0.554

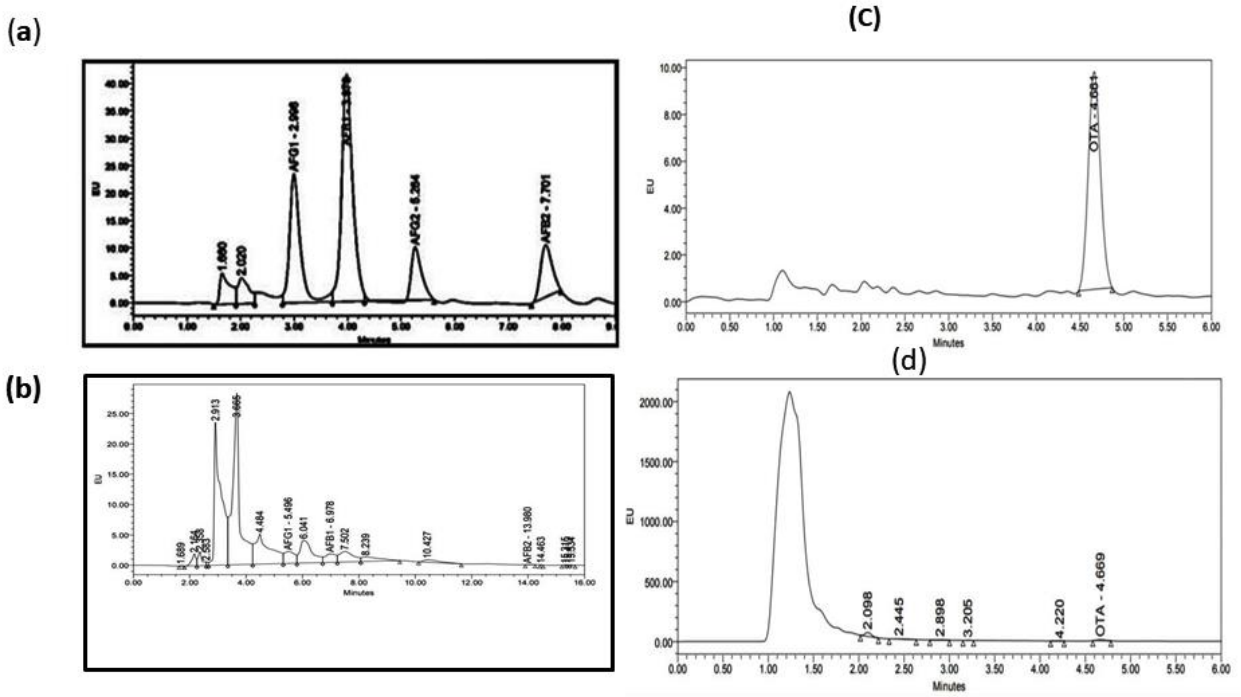

Chart 1. (a) HPLC chromatogram of AFs stander; (b) HPLC chromatogram of AFS produced by *A. flavus var. columnaris* in liquid YES medium; (c) HPLC chromatogram of OTA stander; (d) HPLC chromatogram of OTA produced by *A. niger* van Tieghem in liquid YES medium**.**

CONCLUSION

Most of the examined samples including meat products as basterma, luncheon, burgers, meat, sausage, meat spices, as well as other food products like crisps, karate (snacks), corn flex, biscuits, maize, indomie and soybean were contaminated with different types of fungi that regarded as a major source in food spoilage and can effect on the public health by extraction of their mycotoxins. The production of a wide variety of mycotoxins caused by the majority of fungal genera is regarded to pose serious threats to public health and large financial losses when it comes to the deterioration of meat products.

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