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Occurrences and frequency of fungi isolatedfromfast foods and spices

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Abstract

Meat products especially beef, luncheon and burgers are one of the most popular meals in many countries in the world including Egypt, which were found to behighly contaminated with fungiespecially*Aspergillus and Penicillium* spp during the manufacturing process leading to high economic losses. Also, food spoiling has a public health hazard due to the production of their mycotoxins. Therefore, the current study concentrated on the isolation and identification of mycotoxigenic fungi that were connected to the samples of processed meat So, a total of 54 fast food samples, including corn flex, meat spices, luncheon, burgers, indomie, meat, sausage, crisps, karate (snacks), biscuits, maize and soebean, were gathered from various locations in the Qalubyia governorate in Egypt. The collected samples were examined mycologically. According to the findings of this study,the luncheon samples under examination had the highest total fungus count with 313fungal colonies/10g of the samples, followed by, meat spices (153) and crisps (152)fungal colonies/10g .while indomie, sausage and soebean showed the lowest mean fungus count with (14 , 25 and 25, respectively) and the *Aspergillusniger* was the most frequently and counted 248 in luncheon,98in basterma and 86 in burger followed by *A.flavus* 85 in Crisps, 70 in corn and 66 in meat spices. Because of this, a lot of meat products and spices have severe fungal contamination. These findings indicate the risk of fungal contamination exposure to consumers due to the high consumption offast foods and spiceswhich may be susceptible to fungal infection, leading to mycotoxin contamination if the storage conditions are favorable for fungal growth.

Keywords :meat products, spices, *Aspergillusniger*,*Aspergillusflavus*

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 pathogen.

Consumers may choose to cook them for a better taste or appearance[1]**.**Some ready-to-eat foods were consideredas 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 werecontaminatedduring storage, distribution facilities, soil, water ,airenvironment and human activitiesincludingthefood handlers and vendors[2]. Meat products provides an excellent growth media for a variety of microorganisms[3]**.**

It is believed that there is a big problem with meat products that contaminated with several types of fungi becauseitmakesdecayanddisintegration morelikely,it has aneffect on themeat 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 abad 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 aremore appealing of highly nutritious diets for humansconsumptions. 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, 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 condition, fungal bio-deterioration of stored food is a chronic problemespecially in tropical hot and humid climates due to excretion of mycotoxins [10,11], that can be produced by different fungi such as *Aspergillus, Penicillium, Alternaria, Fusarium, Cladosporium, Mucor,* and *Rhizopus*[12].Somefungi,including*Aspergillus,Fusarium,Penicillium,* and *Alternaria* have thecapacity to cr eatemycotoxins,which harmful byproducts ,that can c ontaminate food under specific conditions[13,14].

According toestimates, more than five billion individuali ngestcontaminated foodseverydayandareexposedtomycot oxinsthroughunidentifiedpathwaysevery day[13].The disease caused by ingestion of Mycotoxin called Mycotoxicosis [15].

The aim of this researchis to evaluate certain (ready-to-eat)food items available in Qalubyia shops in terms of their mycological quality. This mycological study investigates the total fungal counts; isolation and identification of recovered fungal species.

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 markets in Qalubyia Governorate. The food samples from supermarkets were taken to microbiology lab in faculty of science,Benha University in sterile plastic bags in Ice-Box, according to Chessbrough[16].

Usingestablishedtechniquesdescribedin ICMSF[17],thesampleswereexaminedforbacteriaand f ungirelatedtohuman health.

2.2. Sampling preparation

90 ml of 1% peptone water was added to an aseptically prepared blender jar containing 10grams of each sample. The mixture was then homogenized for two minutes in the sterile warring blenderbefore being diluted ten times in serial fashion. The previously made serial dilutionswere inoculated separatelyin one millimeter portions intopetri dish plates and combined with potato dextrose agar medium.

The plates were combined, given time to firm up, and then incubated for 5-7 days at 25°C.

2.3. Isolation and purification of fungi

Transferring a single fungal colony to fresh medium plates on potato dextrose agar resulted in purecultures of colonies [18].

On PDA agar plates, fungal colonies were also cultivated for roughly 7 days at (25°C).

2.4. Cultural and morphological

Based on cultural and morphological characteristics on particular media and availableliterature; slides were continuously examined under multiple powers of microscope, namely 10 and 40X. These characteristics were then compared with the descriptionprovided by another study [19] for the type *Aspergillus,* the isolates of *penicillium* spp. were detectedaccording to Ramirez [20] and Pitt[21].All developing fungus werecultivatedon PDA slants and kept in refrigerator for the genera of imperfect fungiThe formula was used to determine the frequency of fungi and the proportional percentage of each species within a genu s of fungi [22]**.**

Frequency%=Number of samples infected with fungiX100

Total number of sample analysis

Relative Percentage % = Number of fungal spices isolatedX100

Total number of fungi

In Petri dishes with Czapek's yeast extract medium (CYA), fungus isolates were cultivated [23].The media was consisting of (g/L): Sucrose, 30; Na_2NO_3, 2; K_2HPO_4, 1; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4$, 0.01; $ZnSO_4$, 0.01; $CuSO_4$, 0.005; yeast extract; chloramphenicol, 0.25 and agar. Cultures were incubated at 28° C for 7 days. The growing fungi were identified using the colony's features (growth rate, color, texture, and reverse pigmentation) and microscopic information (shape of conidiophores, conidiogenous cells, and conidial dimensions).For easier visualization , lactophenol cotton blue was used to dye fungus hyphae and conidia.

The stain contained 20 g; of phenol crystals ($C_6H_5O_4$), 0.05g of cotton blue (or methylene blue), 40 mlof glycerol, 20 ml of lactic acid (CH_3CHOH COOH), and 20 ml of distilled water. Axiostartrinocular microscope from Zeiss, Germany, was used for microscopic investigation and imaging.A digital camera made in Japan with 7.1

megapixels, the Canon G6, is included withthe microscope[24].

3- Results and Discussion

3.1. The total fungal count of the analyzed processed samples

Data presented in Fig. (1) showed that mycologicalexamination of fourteen processedsamples ,i.e. cornflex, chickenstock, meat spices ,luncheon ,basterma, burger, indomie ,meat, sausage, crisps ,karate (snacks),biscuit, corn and soebean . The greatest total fungal count was found in the luncheon samples, which noted 313 fungal colonies/10g:of the samples under examination ,followed by, meat spices (153) and crisps (152)fungalcolonies/10gwhileThe least mean fungus count was found in samples of indomie,.sausage and soebean, which recorded(14,25 and 25, respectively) fungal colonies/10g .Similar results were found by many researchers [25].

According to their examination of 20 beef burger samples collected from the Assiut, Giza, and Cairo Governorates, the fungus population ranged from l64 to 528 colonies/g.The overall fungal count of the beef samples, according to Omorodion&Odu[26], 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 supportwith[27], who found that the incubation temperature,types of media, and techniques of food analysis are all factors that affect the fungalcounts :However, 92% of the samples of beef luncheon that identified as being contaminated with molds[28].Besides[29]who found that In 70% of the samples of hot dogs, mold was discovered.Different conditions for the relevant meat products (raw or cooked), the quality of the meat and its additives, sanitation during processing and packaging, maintenance of adequate refrigeration from the processor to the retail level and to the consumers, sanitation during handling at the retail stores, and lastly the laboratory technique for detecting fungi contamination tested can all be used to explain the varying percentages of fungi-contaminated samples[30]. By causing the breakdown of the meat's components and the releaseofvarious acids and gases, which changes the meat's odor and flavor, the presence of molds can lead to the spoiling of meatproducts. Some molds are capable of producing mycotoxins, which are hazardous by-products that can cause cancer[31].

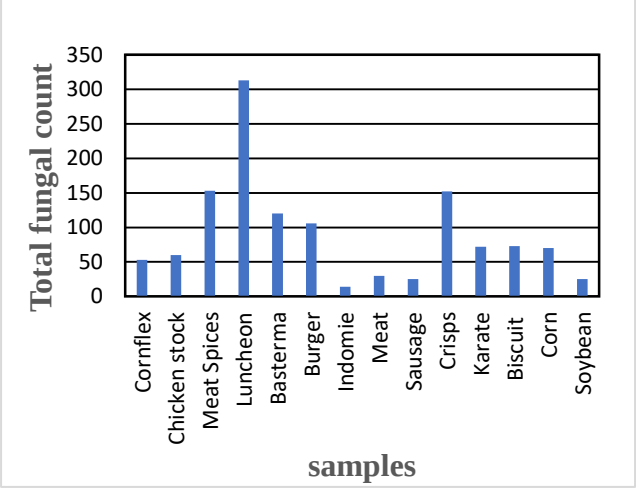

Fig.(1): Total fungal countof the examined processed samples.

3.2. Fungal frequency associated with tested samples:

A complete list of all mycoflora recovered from examined processed samples was provided in Table 2**.** This table's findings showed that seven fungal genera

were recognized and reported. These are *Aspergillus,Penicillium,Mucor,Rhizopus,trichoderma, Fusariumand Alternaria*. The *Aspergillus niger* and *A.flavus* were the genus most detected at high frequency in all samples. The most common species were *A.niger* 87%, *A. flavus* 59.3% followed by *Pencilliums*pp. 25.9 %. Also, The highest relative density percentage was shown by *Aspergillus* species especially, *A.niger* were themost common and isolated with higher density (62.9%) followed by *A.flavus*(31.2%) In this study the fungal genera were recovered, known to be mycotoxigenic according to [32]. 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].

The same results have been achieved by[33] which found the presence of *Aspergillus* spp. *i.e .A.niger* (40%) followed by *A.flavus*(27%) ,*A.ochraceus* (15%),A. *fumigatus* (10%), A.*japonicus* (5%) and *A.sclerotiorum* (3%) in corn.

*A. flavus and A. parasiticus*can contaminate corn while being transported and stored. Due to the fact that both species' spores have a lengthy air survival lifespan and the ability, tospread widely from one place to another [34,35].

On the other hand, the environmental conditionsin the factories, warehouses, freezers, and stores are favorable for the moldsAlthough 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[36].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*[37].Additionally, [38]assessed the microbiological quality of meat products sold in a number of supermarkets and stores in the Gharbia Governorate, including luncheon, basterma, kofta, and burgers, as well as spices (used in meat processing).In 120 analyzed meatproducts and 33 samples of spices, they found 9 different mold genera, including *Aspergillus, Penicillium, Acremonium, Cladosporium, Geotrichum, Mucor, Claveolaria*,the main *Penicillium* species were *P.citrinum*,and *P.aurantigreu*. *P. chrysogenum* was the most frequently isolated species from burger samples, while *P. paxilli* and *P. restrictum* were the most frequently recovered species from kofta samples. Only samples from luncheon and basterma were found to have *p.citreognigrum* and *P. carneum.* The most fungi were found in the luncheon samples, then in the basterma samples, while hawawshi samples had lower mold counts. The fungus genera that were found belonged to the genus *Aspergillus, Penicillium,Cladosporium, Mucor, Eupenicillium, and talaromyces.A. niger, followed by A. flavus and A. parasiticus*, had the highest incidence of isolates *among Aspergillus* species. , according to[39]**.**

Table (1) :Fungal frequency associated with examined samples.

Total fungal species	Total No.of isolates	No.of positive samples	Frequency (%)	Relative density (%)
A.niger	790	47	87.04	62.99

A.flavus	395	32	59.26	31.20
Aspergillusoryzae	19	5	9.26	1.52
A.ochraceous	1	1	1.85	0.08
Pencillium sp.	33	14	25.93	2.63
Mucar	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	2	1	1.85	0.15

3.4 .Occurrence of fungi isolatedfrom the analyzed samples

The results in Table 2 showed that the samples that were infected with mycoflora were grown on PDA media. The total fungal count (TFC) was 1266 isolates. Data also revealed that *Trichoderma, Aspergillus, Pencillium, Fusarium, Rhizopus, and Alternaria*were the most commonly isolated fungus species. It was noticed that *Aspergillus* spp. **(***A.flavus*and*A. niger***)** were the most frequent in all samples. We noticed that *A. niger* was the most frequent and counted 248 in luncheon, 98 in basterma and 86 in burger followed by *A.flavus*. 85 in Crisps, 70 in corn and 66 inmeatspices Also, *A.oryazae* isolates was examined in luncheon that recorded 12 isolates , the predominant *Penicillium*s pecies isolated from cornflex,chicken stock, meat spices, luncheon , basterma, Burger. These results of fungal incidence accept with the results provided by[5,23,33,34]. They discovered that *Aspergillus* spp. and *Penicillium* spp. were the most often isolated fungus from meat products.As well as production areas, shops, andrefrigerators all ideal environments for thegrowth of fungus on meat andmeat products. Which they were highly contaminated with mold especially *Aspergillus* spp and *Penicillium* spp which may gain access during the manufacturing process leading to high economic losses and have a public health hazard due to the production of mycotoxins that create a major economic losses and public health problems.These conclusions resemble those made by [5] who examined beef burgers, sandwiches kofta, oriental sausage, and basterma and discovered nine fungal genera. The genus *Aspergillus* had the highest incidence rate(49%) followed by *Penicillium* (34%), each of *Cladosporium* and *Alternaria*(15%), *Acremonium*(12%),*Rhizopus*(10%), *Rhizomucor*(8%), *Absidia*(3%) and *Chrysosporium*(2%).Also, they foundthat five species of *Aspergillus* could be isolated form meat products samples. *A. niger* had the highest incidence rate(22%) followed by *A.flavus*(16%), *A. fumigatus*(12%), *A. parasitcus* (2%) and at least *A.ochraceus* (1%), this variation in fungal species may be due to using different types of media and storage temperature. according to[35] and [36], because of their shared ability to tolerate in low water activity (0.78-0.83),survive at low to moderate temperatures and thrive on protein-rich substrates.

Table2: Incidence of fungi isolated from the examined samples

Sample/ Mold	*Aflavus.*	*A.niger*	*A.terrus*	*A.paraciticus*	*A.oryazae*	*A.Ochrachaeous*	*Rhizobus sp*	*Alternaria sp*	*Mucar sp*	*Tricoderma sp*	*Fusarium sp*	*Penicillium sp*	Total
Cornflex	4	43	0	0	0	0	0	0	1	0	1	4	53
Chicken stock	55	0	0	0	0	0	1	0	0	0	0	4	60
Meat Spices	66	62	2	0	6	1	3	0	0	0	4	9	153
Luncheon	38	248	0	2	12	0	3	0	1	0	0	9	313
Basterma	12	98	0	0	1	0	0	0	0	4	0	5	120
Burger	14	86	1	0	0	0	1	2	0	0	0	2	106
Indomie	4	10	0	0	0	0	0	0	0	0	0	0	14
Meat	0	30	0	0	0	0	0	0	0	0	0	0	30
Sausage	0	25	0	0	0	0	0	0	0	0	0	0	25
Crisps	85	66	0	0	0	0	1	0	0	0	0	0	152
Karate	5	66	0	0	0	0	1	0	0	0	0	0	72
Biscuit	42	31	0	0	0	0	0	0	0	0	0	0	73
Corn	70	0	0	0	0	0	0	0	0	0	0	0	70
Soebean	0	25	0	0	0	0	0	0	0	0	0	0	25
Total	395	790	3	2	19	1	10	2	2	4	5	33	

Identification of fungal isolates

AS shown in photo (1),
the most prevalent isolates were determined based on preliminary morphological and cultural properties at Assiut University Mycological Centre (AUMC), Egypt with deposition numbersAUMC16127, AUMC16128,AUMC16129 and AUMC16131for*Aspergillusoryzae*,*Aspergillusflavus*v arcolumnaris,*Aspergillusniger* van tieghem and *Penicillium* verrucosum,respectively. Most of the testedmeat product samples were infected with different types of fungi which considered as a major cause in the spoilage of meat products, leading to high economic losses and constitute a public health hazard by the production of a wide variety of mycotoxins. Data shows that some tested processed meat samples, i.e. basterma, beef burger, Luncheon meat and sausage were found to be highly contaminated with fungi including *A.oryzae*, *A.flavus* var columnaris,, *A.niger* van tieghem and *Penicillium* verrucosum. *Aspergillus* spp was the most spoilage fungi isolated from most type of foods and this result are similar to the experiments that done byEasa[40] which isolated different *Aspergillus* species from traditional fast foods. This result showed that themost important fungal species belonging to *Aspergillus flavus* and *Aspergillus niger,* which are mycotoxin-producers

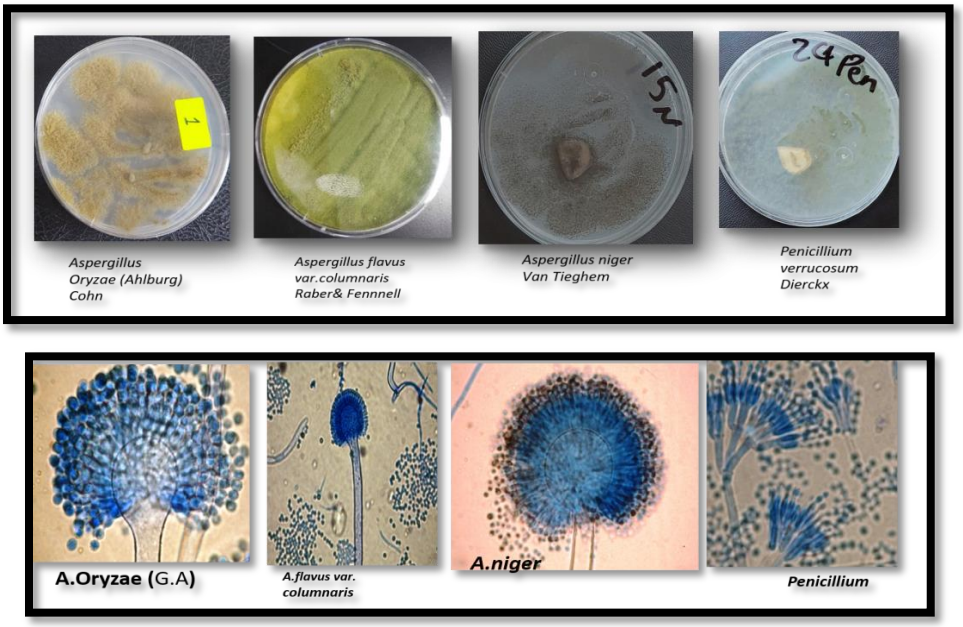

Photo (1): Fungal genera isolated from the samples

CONCLUSION

Most of the examined samples including meat products as (beef,basterma,corn flex, meat spices, luncheon, burgers, indomie, meat, sausage, crisps, karate (snacks), biscuits, maize and soebean)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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