Mycological Studies On Fresh And Frozen Sausage With Particular Reference To Aflatoxin Contamination

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ABSTRACT

A total of 75 samples namely, 50 fresh sausages from different butcher's shops and 25 frozen sausages were collected from supermarkets in Alexandria Governorate. The mycological examination of these samples revealed highest rate of mould and yeast contamination, 46% for fresh sausages, then frozen sausages samples, which had the lowest level of contamination (24%). The members of *Aspergillus* species were the most predominant species isolated (30%) and (28%), followed by *Penicillium* species (28%) and (8%) then *Fusarium* (6%) and (0%), *Alternaria* (4%) and (0%), *Cladosporium* (4%) and (8%), *Trichoderma* (0%) and (4%), *Mucor* (4%) and (4%), *Candida* (6%) and (0%) and *Saccharomyces* (10%) and (8%) for fresh sausages and frozen sausages, respectively. The highest incidence of isolation of *Aspergillus* species were recorded in *Aspergillus flavus* (14%) and (18%) for fresh sausages and frozen sausages, respectively. Significant levels of aflatoxins particulary aflatoxin B1 and B2 were detected in examined samples, the maximum rate is observed in fresh sausages (43%) 3 samples out of 7 were toxigenic strains. While, the lowest rate is detected in frozen sausages (25%) 1 samples out of 4 were toxogenic strains. aflatoxin B1 and B2 were detected in examined samples 46.5 µg/L and 11.98 µg/L, 25.74 µg/L and 0 and 18.29 µg/L and 3.75 µg/L for fresh sausages, respectively. While, 6.17 µg/L and 2.25 µg/L were recorded for frozen sausages, respectively. The health significance of isolated fungi and mycotoxins in fresh and frozen sausages was fully discussed.

INTRODUCTION

In recent decades, the progressive increase in requirement of meat products for human consumption resulted in an intensive attention of its hygienic status. The question of mould toxicity has attracted attention, especially in the fields of food industry *(1)*.

Fungi comprise a large group of microorganism, which are ubiquitous in nature and contaminate meat products as a result of food deterioration in developing countries. The growth of fungi in food is regarded as an indicator for the presence of mycotoxins (fungal toxic metabolites) leading to a food borne mycotoxicosis *(2)*.

Sausages are products in which fresh comminuted meats are modified by various processing methods to yield desirable organoleptic and keeping properties. It is traditionally stuffed into casings but can be formed into patties and fried like hamburgers. It is made up of ground meat and spices. As the user controls both, the variations are endless.

Sausages are one of the oldest forms of meat processing and modern sausage technology has its roots deeply embedded in history. Fresh sausages are made from fresh meats which are, as a rule, neither cured, smoked, fermented nor cooked. Fresh sausages are kept only a few days in the refrigerator but can be frozen for future use. They are heated by the consumer before serving. Frozen sausage are cooked, either in hot water or a smokehouse and will keep under refrigeration for months in addition to the enhancement of long keeping qualities *(3)*.

The conditions of the environment in the manufacturing rooms, stores, refrigerators and shops are very suitable for the development of moulds inside the products, but more frequently on the surface of sausage *(4)*. The mould growth

on foods stored at low temperature is a common and reoccurring problem. They are able to produce mycotoxins at temperature as low as -2 to -10°C *(5)*.

Mould growth on meat products can take place not only during processing but also during wholesale distribution and in retail showcase. At each stage in the distribution chain, pigmented mould is removed by washing to improve product appearance. The danger inherent in the practice of mould removal by washing is that the procedure does nothing to remove toxic and allergic mould metabolites which may have diffused into the meat *(6)*.

In Egypt, during the last three decades mycotoxicosis caused great economic losses to meat production in the form of impaired performance and mortalities *(2)*. Aflatoxins are natural contaminants in many types of food and are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* . Aflatoxins are hepatotoxic and hepatocarcinogenic to man *(7)*.

Howevre, the harmful effect of mycotoxicosis is attributed to the interruption of protein synthesis and interference with carbohydrate and fat metabolism, which lead to either food poisoning or spoilage of food and reduces its quality *(8 , 9)*.

Humans are exposed to aflatoxins by consuming foods contaminated with products of fungal growth, such exposure is difficult to avoid because fungal growth in foods is not easy to prevent, still remains for the possible adverse effects resulting from long term exposure to low levels of aflatoxins in the food supply. The aflatoxins problem has been reported to be more serious in tropical and subtropical regions of the world where climatic conditions of temperature and relative humidity favour the growth of moulds and aflatoxine production *(10,11)*.

Aflatoxins B1 and B2 have received greater attention than any other mycotoxins because they classified as Group1 human carcinogens *(12)*.

The consumption of meat contaminated by moulds as well as mycotoxine induces haemorrhage, hepatotoxicosis, nephrotoxicosis, neurotoxicosis, dermatotoxicosis, genotoxicosis, teratoxicosis carcinogenesis or have hormonal and immunosuppression effects *(5, 13)*.

Therefore the this study was carried out for evaluation of fungal contamination as well as detection of some mycotoxins in fresh and frozen sausages.

MATERIAL AND METHODS

1.Collction of samples

A total number of 75 samples namely, 50 fresh sausage were collected from different butcher's shops and 25 frozen sausage were collected from different supermarkets in Alexandria Governorate in clean, dry and sterile polyethylene bags and transferred to the laboratory for mycological examination.

2.Isolation of moulds and yeasts

Samples were prepared and examined for isolation of fungi according to the recommended technique *(14)*, five grams of finely ground sausage were added to 45 ml of Sabouraud dextrose broth in stomacher jar(original suspension). One ml from the original suspension already prepared was transferred to a test tube containing 9 ml of sterile Sabouraud dextrose broth and thoroughly mixed to have a dilution of 1/100. Then, ten fold dilutions were prepared according to the required dilutions. One ml from each dilution was poured into duplicate sterile Petri dishes, then the melted and tempered Sabouraud dextrose agar were added. The plates were left to solidify at room temperature then incubated at 25C for 5-10 days.

3.Identification of moulds and yeasts

After the end of incubation periods, the isolates were identified with genera Aspergellus speciesr according to the recommended technique *(15)*.

4.Aflatoxin examination of samples

The aflatoxins were extracted from samples and their levels were measured by thin layer chromatography (TLC) *(16,17)*.

RESULTS

Table 1. Incidence of contaminated samples with mould and yeast genera in fresh and frozen sausages .

Product	No. of samples	Positive samples		Negative samples	
		No.	%	No.	%
Fresh sausage	50	23	46%	27	54 %
Frozen sausage	25	6	24%	19	76%

No.=Number of samples.

Table 2. Incidence of mould and yeast genera isolated from the examined different samples of fresh and frozen sausage (n = 25).

Type of fungi	Fresh sausage (No.=50)		Frozen sausage (No.=25)	
	No.	%	No.	%
Mould				
Aspergillus	15	30%	14	28%
A. flavus	7	14 %	4	16 %
A. terrus	2	4 %	1	4 %
A. ochraceus	2	4 %	1	4 %
A. versicolor	0	0	1	4 %
A. fumigates	2	4 %	0	0
A. nidulans	2	4 %	0	0
Penicillium spp.	14	28 %	2	8 %
Fusarium spp.	3	6 %	0	0
Alternaria spp.	2	4 %	0	0
Cladosporium	2	4 %	2	8 %
Trichoderma	0	0	1	4 %
Mucor	2	4 %	1	4 %
Yeast				
Candida	3	6 %	0	0
Saccharomyces	5	10 %	2	8 %

No.=Number of examined samples . A= Aspergillus.

Table 3. Screening of *Aspergillus flavus* by TLC isolated from examined sausages for production of Aflatoxin in YES medium (ug/L medium).

Type of product	Number of *A.Flavus* strains	Toxogenic strains No.	%	B1	B2
Fresh sausage (No=50)	7	3	43%	46.5 µg/L 25.74 µg/L	11.98µg/L ------
Frozen sausage (No=25)	4	1	25%	18.29µg/L 6.17µg/L	3.75 µg/L 2.25µg/L

No.=Number of examined samples.

DISCUSSION

Due to the seriousness associated with fungal contamination of food, the mould and yeast are considered a standard test for checking the general sanitary hygiene conditions during meat processing *(2)*.

Regarding the results recorded in Table 1 the incidence of contaminated samples with mould and yeast genera in fresh and frozen sausages were declared that fresh sausages samples had the highest rate of mould and yeast contamination (46% than frozen sausages samples), which had the lowest level of contamination (24%). Higher results were obtained several authers *(9,18-21)*.

Butcher's shops leave fresh sausages hanging without packing for nearly about 24 hours may be inside or out side their shops or at street to pay attention consumer see and buy it, this habits give the chance to high mould and yeast contamination than the frozen sausages which present in refregrations at low temperature and had different types of packing.

Contaminations of meat products either fresh or frozen by moulds are common due to the ubiquitous distribution of mould spores. Contaminations start with slaughtering. The environment inside the slaughter halls including air movement, walls, floor, hides and intestinal contents of the slaughtered animals play the main role in contamination of meat with moulds *(22)*, and unhygienic handling during processing of sausages.

The main causes for the growth of moulds in meat products include an inadequate process of drying resulting in a high water activity within the whole products or only on the surface of the products, as well as unsuitable storage conditions .The presences of various moulds in sausage were due to added flavorings to meat during production of sausage which was heavily contaminated with moulds. The widest spectrum and the highest counts of microscopic filamentous fungi were observed in the following spices: milled black, pepper, nutmeg, garlic powder and crushed caraway. The level of contamination depended upon the season, being higher in the summer months *(23)*.

The significant increase in yeast and molds in sausages may be attributed to the contribution from other ingredients such as starch and spices *(24)*.

in frozen sausage the prevalence of these species in cold stores was attributed to their ability to grow at low temperature *(25)*.

The current results in Table 2 indicated that the members of *Aspergillus* species were the most predominant species isolated (30% and 28%), followed by *Penicillium* species (28% and 8%) then *Fusarium* (6% and 0%), *Alternaria* (4% and 0%), *Cladosporium* (4% and 8%), *Trichoderma* (0% and 4%), *Mucor* (4% and 4%), *Candida* (6% and 0%) and *Saccharomyces* (10% and 8%) for fresh sausages and frozen sausages, respectively. The highest incidence of isolation of *Aspergillus* species were recorded in *Aspergillus flavus* (14% and 18%) for fresh sausages and frozen sausages, respectively. Higher values were recorded in several previous studies *(9, 18-20)*.

The spores of moulds are ubiquitously spread in the environment and can be detected everywhere. *Aspergillus* and the *Penicillium* species were the most frequently isolated genera of molds from sausages *(26, 27)*.

The incidence of moulds in sausages was relatively high due to added salts which make meat more favorable for growth of moulds where *Aspergillus* and *Penicillium* species have the ability to tolerate the high concentration of salts *(28)*.

Both potassium sorbate and pimaricin, also known as natamycin have been found effective as antifungal treatments on meat products which eliminate or reduce the surface molding problem *(6)*.

The current data present in Table 3 revealed that the significant levels of aflatoxins particularly aflatoxin B1 and B2 were detected in examined samples, the maximum rate is observed in fresh sausages (43%), 3 samples out of 7 were toxogenic strains. While, the lowest rate is detected in frozen sausages (25%) 1

samples out of 4 were toxigenic strains. aflatoxin B1 and B2 were detected in examined samples (46.5 µg/L and 11.98 µg/L), (25.74 µg/L and 0) and (18.29 µg/L and 3.75 µg/L) for fresh sausages, respectively. While, 6.17 µg/L and 2.25 µg/L were recorded for frozen sausages , respectively. Nearly similar results for frozen sausage were obtained *(29)* they found (7 µg/L B1 and 3 µg/L B2). Higher results were cited *(5,9,18,20)* .

Fresh sausages of high humidity above 70% was a favorable substrate for aflatoxin production when kept at 20-30°C. Aflatoxin may introduced to the meat or meat products either by direct contamination resulting from growth of aflatoxin producing mould or indirectly through feeding of meat producing animals on moldy feeds or through the use of contaminated meat additives and spices, which are added to improve the meat product quality *(20)*

The contamination of meat products by fungi and aflatoxins constituted a public health hazard. The lack of hygienic measures during slaughter, processing and storage of meat and meat products as well as the added spices and some food additives were considered as the main source of toxigenic moulds and mycotoxins which lead to either food spoilage or food borne mycotoxicosis *(9)*.

Aflatoxin production is favored by temperature of 20 to 25°C but has been reported to occurs as low as 7 to 12 °C *(30)*.

The production of aflatoxin by *Aspergillus flavus* was controlled by oxygen and NaCl requirements which increase the mould growth and enhance the production of aflatoxin *(31)*.

Aflatoxine can induce DNA damage and mutation, toxicity resulting in cell death and a consequence increase in cell replication and immunosuppression *(32)*.

Continuous exposure with low level of aflatoxins enhance the susitability to susceptibility and tumorigenesis *(33)*.

The liver is the principal target organ for aflatoxin B1 and a high incidence of human hepatic and extrahepatic carcinogenesis occurs in areas with high endemic aflatoxin B1 concentration in food *(34)*. So, preventing the mould growth is the best method to stop toxin production this may be attained by preventing the natural contamination of raw materials.

Finally, the regulatory limits could not prevent chronic effect of aflatoxin hazards due to the cumulative effect of repeated exposure to very small or even undetectable doses of aflatoxins for long period in man and animals.

Accordingly, it is recommended that continuous monitoring survey to detect mycotoxins in food should be applied. Application of basic hygienic and environmental storage requirements of food for keeping quality, including moisture, temperature ,freshness and clean equipments and using of mould inhibitors .Sanitary measures adapted in handling of fresh raw meat or fresh sausages during prolonged storage, transportation, packing, marketing, during preparation of minced meat, processing and quality of spices or meat additives used.

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الملخص العربى

دراسات فطرية على السجق الطازج و المجمد مع الإشارة الى التلوث بسموم الافلاتوكسين

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أصبحت منتجات اللحوم عرضة للتلوث من مصادر مختلفة بالفطريات سواء كانت من البيئة المحيطة او اثناء مراحل التصنيع المختلفة حتى اثناء الحفظ و التوزيع غالبا ما تتلوث بها و تفرز سموم فطرية تسبب أضرارا على الصحة العامة , لذلك اجريت هذة الدراسة على عدد ٧٥ عينة من كل السجق الطازج المنتج فى محلات الجزارة(٥٠ عينة) و السجق المجمد المنتج فى المصانع و المجمع من المحلات و السوبر ماركت (٢٥ عينة) بمحافظة الأسكندرية,تم فحص هذة العينات من حيث مدى تلوثها بالفطريات و الخمائر و سموم الافلاتوكسين و قد دلت نتائج الدراسة على ان اعلى نسبة تلوث بالفطريات و الخمائر كانت فى السجق الطازج بنسبة (٤٦%) يليه السجق المجمد بنسبة(٢٤%) , كما تم عزل اصناف الاسبرجلس بنسبة كبيرة عن باقى اصناف الفطريات المعزولة(٣٠%) و(٢٨%)لكل من السجق الطازج و المجمد على التوالى , يليه اصناف البينيسيليوم (٢٨%) و (٨%) , الفيوزاريوم (٦%)و(٠%), الترناريا (٤%) و(٠%),الكلادوسبوريوم (٤%) و(٨%), ترايكوديرما (٠%) و(٤%) والميوكر(٤%) و(٤%) , ومن أصناف الخمائرالمعزولة الكانديدا(٦%) و(٠%) و سكاروميسز(١٠%) و(٨%) لكل من السجق الطازج و المجمد على التوالى.تم تحديد مستوى تلوث العينات بالسموم الفطرية خاصة نوعى ب١ و ب٢ و قد وكانت النسبة الاعلى للتلوث فى السجق الطازج (٤٣%) اى ٣عينة من٧عينات موجبة للسموم الفطرية بينما كانت نسبة التلوث بالسموم الفطرية فى السجق المجمد اقل (٢٥%) اى ١عينة من٤عينات تحتوى على سموم فطرية.وقد تم تحديد السموم الفطرية نوعى ب١ و ب٢ كالأتى (٤٦,٥% و ١١,٩٨%µg/L) , (٢٥,٧٤% µg/L.) و (١٨,٢٩% و ٣,٧٥%µg/L) فى السجق الطازج على التوالى , بينما كانت للسجق المجمد (٦,١٧% و ٢,٢٥%µg/L) على التوالى, هذا و قد تمت مناقشة الخطورة الصحية لهذة المعزولات مع بيان المصادر المختلفة للتلوث بتلك الملوثات الخطيرة.