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Microbiological Quality of Some Meat Products in Local Markets with Special Reference to Mycotoxins

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Abstract: The aim of this work was to evaluate the microbial quality of meat products sold in local markets at Cairo and Giza provinces and to investigate their hygienic significance. Samples were subjected to mycological, bacteriological, aflatoxin B_1 (AFB_1), analysis for evaluation their quality and safety. The incidence of mould and yeast in the examined meat product samples were 16 (64%), 22 (88%), 23(92%), 7(28%), 22(88%) and 5 (20%) for Frankfurter, Luncheon and Basterma respectively. In the examined samples, 7 mould genera could be identified. The identified mould genera were Aspergillus, Pencicillium, Eupencillium, Eurotium, Mucor, Cladosporium and Byssochlamys nivea. The predominant species were Aspergillus, Penicillium and Mucor. While the identified yeast genera in the examined frankfurter samples were; *C. parapsilosis* 7(50.0%), *Rhodotorula mucilaosagin* 3(21.4%), *C. krusei* 2 (14.3%) and *Geotrichum candidum* 2 (14.3%). The frequencies of isolated yeast genera in examined luncheon samples were; *C. krusei* and *C. parapsilosis* 5 (38.5%), *Rhodotorula mucilaosagin* 2 (15.4%) and Torulopsis species 1 (7.6%).While in basterma samples the frequencies were; *C. parapsilosis* 8 (38.1%), Torulopsis species 8 (38.1%) and *C. tropicalis* 5 (23.8%). The obtained results of AFB_1 in examined samples revealed that 5 (20%) samples of luncheon contain aflatoxin B1 minimum 1.3, maximum 24.5 and average ± SE 10.4± 5.1ppb, while the value of aflatoxin B1 in Basterma samples minimum 1.2, 2.5 and average ± SE 2.3± o.4 ppb. Aflatoxin B_1 was not detected in Frankfurter examined samples. Bacteriological analysis revealed that the incidences of *S. aureus* and *Cl. perfringens* were 28 and 20 % in Frankfurter samples, while in Luncheon were 32 and 32%, respectively. In Basterma samples the incidence represented in 40 and 24% for *S. aureus* and *Cl. Perfringens* respectively. 7 and 8 strains of *S. aureus* isolated from frankfurter samples and Luncheon were examined against enterotoxins production and typing and the results showed that 2 and 1 strains are enterotoxigenic and produce enterotoxins C. type.

Key words: Meat Products · Mould · Yeast · Aflatoxin B_1 · *Cl. perfringens* · *S. aureus* · Enterotoxins C. Type

INTRODUCTION

Microorganisms control in meat products is the major concern in the preparation of high quality foods. During slaughtering process the meat is exposed to many sources of contamination [1]. The hygienic state of animals prior, during and after slaughter can be critical to the finished product quality [2].

During the deboning process the meat undergoes extensive handling and is susceptible to bacterial contamination resulting in pigment decomposition, discoloration and development of off odors [3].Well known bacteria implicated in food borne illness are staphylococcus aureus [4] which are natural habitants of plants and animals but can contaminate foods and cause illness in humans when ingested [5].

Staphylococcus aureus is a facultative anaerobe, non-motile, spherical and Gram positive bacterium. Nausea, vomiting, retchins, abdominal cramping and prostration are the most common symptoms of *S. aureus*

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food poisoning [6], it can be transferred to meat from various sources such as skin of the animal, hide equipment and infected personal [7].

Clostridium perfringens are potentially pathogenic microorganisms that are often contaminants in fresh meat. They are strictly anaerobic bacteria that may be present in the normal gut flora of animals and humans. They are spore-forming bacteria enabling them to survive in unfavorable environments, which present a challenge in food preservation. *Cl. perfringens* poisoning is one of the most common foodborne disease, however presumably with most cases never recorded because of mild and self-limiting disease. *Cl. perfringens* poisoning is caused by an ingestion of a large amount of vegetative bacteria. In the first part of the gut, during sporulation, enterotoxin is released in the gut causing diarrhea, sometimes accompanied by stomach cramps, but usually mild and self-limiting. Symptoms occur 8-24 hr. after ingestion of the meal [8].

Mould contamination of meat and meat products may occur during slaughtering of the animals, transportation, or during processing of meat products through the use of contaminated equipments or contaminated additives and spices are considered the most important source of mould contamination in meat products [9-11].

Mycotoxins are toxic substances elaborated by fungi. They constitute a heterogeneous group of secondary metabolites with diverse potent pharmacological and toxic effects in humans and animals. Most important mycotoxins produced by moulds belong to Aspergillus, Penicillium and Fusarium genus [12-14]. These molecules are usually classified depending on the fungal species that produce them. Certain mycotoxins are considered as carcinogenic or suspected to have carcinogenic properties [15]. However, human consumers may be exposed to these toxic compounds indirectly due to the presence of residual contamination in foods prepared from animals that have been fed with contaminated feeds. Depending on the metabolic pathways involved, the residues may correspond to the native toxin or to metabolites that keep all or part of the toxic properties of the parental molecule. Therefore, the passage through an ‘‘animal filter’’ may represent a detoxification process or, on the contrary, lead to the appearance of more toxic compounds for the human consumer. The exposure of human consumers may also result in the mycotoxin synthesis during ripening of products. Indeed, several studies have shown that mould species belonging to the genus Penicillium and Aspergillus could be isolated from meat products such as ripened sausages or dry cured ham [16, 17].

Therefore, this investigation was planned for evaluation of the microbiological quality of meat products as well as detection of Staphylococcal enterotoxin, *Cl. Perfringens* toxin and aflatoxinB_1 content residues and effect of some food processing methods on the stability of mycotoxin aflatoxinB_1 to heat.

MATERIAL AND METHODS

Samples Collection: A total of seventy five samples of frankfurter, luncheon and basterma (25 of each) were collected from grocery shops and supermarkets. These samples were obtained and preserved in an ice box then transferred to the laboratory under complete aseptic condition without undue delay and examined as rapidly as possible.

Bacteriological Examination:

- Preparation of samples for bacteriological examination [18]
- Isolation of *Staphylococcus aureus*: The isolation was carried out according to technique recommended by FDA [19] using Baird Parker medium incubated at 35°C for 48 hrs.
- Isolation of *Cl. perfringens*: The method was applied according to ISO (7937:2004) [20] and identification according to Buchanan and Gibbons [21].
- Detection of enterotoxin of *Staphylococcus aureus* produced by isolated strains using subcultural method described by Donnelly *et al.* [22].
- Detection and typing of enterotoxins according to Oda Ohkuboty *et al.* and Shingaki *et al*. [23, 24] by reversed passive latex agglutination technique using Oxoid SET-RPLA (Kits used for the detection of staphylococcal enterotoxins A,B,C and D).

Fungal Isolation and Identification: The collected samples were prepared according to the technique recommended by ISO [25]. The isolated fungi were identified according to macro and microscopic characteristics as described in Pitt and Hoching [26], while yeast isolates according to Kriger Van Rij [27] and Tibor and Larry [28].

Detection of Aflatoxin B_1 Residues: The samples were analyzed for aflatoxin B_1 using a slightly modified immunoaffinity method based on Association of Official Analytical Chemists (AOAC) method [29].

RESULTS AND DISCUSSION

Table (1) shows that the incidence of *S. aureus* and *Cl. perfringens* were 28 and 20 % in Frankfurter samples, while in Luncheon was 32 and 32%, respectively. In Basterma samples the incidence represented in 40 and 24 for *S. aureus* and *Cl.* Perfringens, respectively. In this respect, many researchers' studies of isolated food poisoning microorganisms in different meat products were mentioned as Farid [30] who reported that incidence of *S. aureus* 20 % in sausage.

Shaltout [31] isolated *S. aureus* in rate of 18 % while Eleiwa [32] reported that incidence of *S. aureus* 24 % and 16 in luncheon and sausage while *Cl. perfringens* 12 and 20 % respectively.

Torky [33] found that the incidence of *S. aureus* was 15 and 5% and *Cl. perfringens* 25and 30 % in luncheon and basterma samples, respectively. Gergis [34] reported that high incidence of *Cl. perfringens* in Frankfurter, luncheon and basterma 65, 65 and 60 %, respectively. Zaki and Shehata [35] found the incidence of *S. aureus* 26.66 % and *Cl. perfringens* 26.66 %.

The source from which *S. aureus* enter the foods in nasal passages and the infected wound of many persons may be a common source [36]. The ingestion of meat or food contaminated with more than 100 cells of *Cl. perfringens* /g is considered to be unfit for human consumption and lead to poisoning [37]. Most food poisoning cases involving *Cl. perfringens* are reported from restaurants, hospitals and homes for elderly people, through proper cleaning and disinfections, it should be relatively easy to control food borne disease caused by *Cl. Perfringens* [38].

The incidence of enterotoxingenic strain of *S. aureus* and their enterotoxins in table (2) showed that 7 and 8 strains of *S. aureus* isolated from frankfurter samples and Luncheon respectively were tested for their enterotoxins production and typing, as a result 2 and 1 strains considered were enterotoxingenic and could produce enterotoxin C. type, while we can´t detect any toxigenic *S. aureus* strains from basterma. These results are nearly similar to those several authors [35, 39, 40, 41].The risk to public health arises if toxigenic strains of *S. aureus*

Table 1: Incidence of isolated microorganisms from meat products (n=25)

	Samples					
	Frankfurter		Luncheon		Basterma	
Microorganisms	No.	%	No.	%	No.	%
S. aureus	7	28	8	32	10	40
Cl. perfringens	5	20	8	32	6	24

Table 2: Enterotoxin types of isolated S. aureus

Examined samples	No. of isolated strain	Enterotoxigenic strain	Types of Enterotoxins
Frankfurter	7	2	C
Luncheon	8	1	C
Basterma	10	-	-

multiply to great numbers during improper handling and storage as a result extracellular compounds produced from which enterotoxins (exotoxigens) responsible for the symptoms of staphylococcal food poisoning which are mostly common as nusea, vomiting and diarrhea [42].

In uncured, cooked meats such as roasts in which the temperature reaches only below 100°C in the center, *Cl. perfringens* is of the most concern due to the ability of its spores to survive the heat treatment, germinate and proliferate during post-cooking handling [8]. Related outbreaks occur only after post-cooking temperature abuse. There are no accompanying spoilage species to make the meat inedible or ''warn'' consumers. Furthermore, *S. aureus* producing heat stable enterotoxin before cooking is associated meat safety concerns in these products [8].

The typical scenario for staphylococcal food poisoning is by contamination of a heat-treated food, through handling by personnel, followed by a temperature abuse. Heating will destroy most of the competing flora, which together with cooling failure will provide ideal conditions for growth of staphylococci, should the food by accident or malpractice be contaminated. Sufficient amounts of enterotoxin is required to cause food poisoning that *S. aureus* have been growing to relatively high numbers in the food, about 10^6[8].

The results achieved in table (3) revealed that the incidence of mould and yeast in the examined meat product samples were 16 (64%), 22 (88%), 23(92%), 7(28%), 22(88%) and 5 (20%) for Frankfurter, Luncheon and Basterma respectively. The results obtained for Frankfurter, Luncheon and Basterma are similar to that recorded by many investigators [43, 44, 45, 46, 47].

Table 3: Incidence of moulds and yeasts in examined meat products (N= 25)

	Positive samples			
	Mould		Yeast	
Meat products	No.	%	No.	%
Frankfurter	16	64	22	88
Luncheon	23	92	7	28
Basterma	22	88	5	20

N= number of samples

Table 4: Frequency percentages of the isolated mould genera in the examined meat product samples

	Frankfurter		Luncheon		Basterma	
Mould genera	No.	%	No.	%	No.	%
Aspergillus species *A.niger*	2	15.4	10	26.3	5	17.3
A. flavus	4	30.8	7	18.4	7	24.2
A.ochraceus	1	7.7	-	-	2	6.9
A.gluacus	-	-	-	-	2	6.9
Pencicillium species						
P. simplicissimum	2	15.4	2	5.3	1	3.4
P. chrysogenum					1	3.4
P. funiculosum	1	7.7	-	-	-	-
P. nalglovense	2	15.4	-	-	-	-
P. digitatum	-	-	2	5.3	-	-
P. corylophilum	-	-	7	18.4	-	-
P. raistrickii	-	-	-	-	1	3.4
P. rugulosum	-	-	-	-	1	3.4
P. olsonii	-	-	-	-	1	3.4
Eupencillium species	-	-	2	5.3	-	-
Eurotium species						
E. chevalier	1	7.7	-	-	-	
Mucor	-	-	5	13.1	5	16.3
Cladosporium species	-	-	2	5.3	3	10.4
Byssochlamys nivea	-	-	1	2.6	-	-

The higher incidence of moulds in luncheon samples were attributed to the use of different untreated food additives and spices which may be the main source of mould contamination in meat products [11].On the other hand, the lower incidence of moulds in basterma and Frankfurter samples may be attributed to the lower water activity (a_w) in these products and presence of garlic [47].

The data obtained in table (4) declared that 7 mould genera could be identified in the examined samples. The identified mould genera were Aspergillus, Penicillium, Eupencillium, Eurotium, Mucor, Cladosporium and Byssochlamys nivea. The predominant species were Aspergillus, Penicillium and Mucor. In Frankfurter the isolated moulds were *A. flavus* 4 (30.8%), *A.niger* 2(15.4%), *P. simplicissimum* 2 (15.4%), *P. nalglovense* 2(15.4%) and *A. ochraceus*, *P. funiculosum* and *E. chevalier*1 (7.7%) for each. The frequencies of isolated mould genera in examined luncheon samples were; *A. niger* 10 (26.3 %), *A. flavus* 7 (18.4%), *P. corylophilum* 7 (18.4%), Mucor 5 (13.1%), *P. simplicissimum*, *P. digitatum*, Eupencillium species and Cladosporium species 2 (5.3%) and *Byssochlamys nivea* 1 (2.6 %). For basterma samples the frequencies were; 7 (24.2%), 5 (17.3%), 5 (16.3%), 3(10.4%), 2 (6.9%), 2 (6.9%), 1 (3.4%), 1 (3.4%), 1 (3.4%), 1 (3.4%) and 1 (3.4%) for; *A. flavus*, *A. niger*, Mucor, Cladosporium species, *A. ochraceus, A. gluacus, P. simplicissimum, P. chrysogenum, P. raistrickii, P. rugulosum* and *P. olsonii.* respectively.

The results of mould identification declared that the most predominant mould genera in meat products samples were; Aspergillus and Pencicillium species which agree with the results obtained by many researchers [44, 48,49,50,51 and 52]. The presence of such moulds and yeasts may cause spoilage of meat products by breaking

Table 5: Frequency percentages of the isolated yeast genera in the examined meat product samples

	Frankfurter		Luncheon		Basterma	
Yeast genera	No.	%	No.	%	No.	%
Candida species *C. krusei*	2	14.3	5	38.5	-	-
C.parapsilosis	7	50.0	5	38.5	8	38.1
C. tropicalis	-	-	-	-	5	23.8
Rhodotorula mucilaosagin	3	21.4	2	15.4	-	-
Torulopsis species	-	-	1	7.6	8	38.1
Geotrichum candidum	2	14.3	-	-	-	-

Table 6: Determination of aflatoxin B_1 in meat product samples

		Aflatoxin positive samples		Amount of AFB_1(ppb)		
Type of meat product	No. of samples	No.	%	Min.	Max.	Average± SE
Frankfurter	25	ND	ND	-	-	-
Luncheon	25	5	20	1.3	24.5	10.4±5.1
Basterma	25	4	16	1.2	2.5	2.3±o.4

down their components and liberating different acids and gas with subsequent change of their odour and flavour. Moreover, mould growth on meat products causes economic losses from discolouration, poor appearance and off flavours; In addition, some moulds are capable of producing toxic metabolites known as mycotoxins such as aflatoxins which are known carcinogenic [26].

Table (5) revealed that the identified yeast genera in the examined frankfurter samples were; *C. parapsilosis* 7 (50.0%), *Rhodotorula mucilaosagin* 3(21.4%), *C. krusei* 2 (14.3%) and *Geotrichum candidum* 2 (14.3%). The frequencies of isolated yeast genera in examined luncheon samples were; *C. krusei* and *C. parapsilosis* 5 (38.5%), *Rh. mucilaosa*gin 2 (15.4%) and Torulopsis species 1 (7.6%).While in basterma samples the frequencies were; *C. parapsilosis* 8(38.1%), Torulopsis species 8 (38.1%) and *C. tropicalis* 5 (23.8%). The results of yeast identification declared that the most predominant yeast genera in meat products samples agree with the results obtained by many investigators [26, 50, 53, 54].

Many studies demonstrated that fungal mycoflora of dry cured meat products is usually complex and made of many fungal species, from which several may be toxigenic, at least *in vitro*. Therefore, the contamination with toxigenic strain may lead to mycotoxins synthesis and accumulation in the final product [8].

Aflatoxins are the most documented mycotoxins. Several studies indicated that dry cured meats can be contaminated with toxigenic *A. flavus* strains, especially when products are processed in countries with hot climate [55]. Moreover, it has been demonstrated that the processing conditions during ageing of hams may allow aflatoxin synthesis. Therefore; it is of public health importance to evaluate the possible production of aflatoxin B_1 during meat processing and ageing.

The results recorded in table (6) revealed that 5 (20%) samples of luncheon are contained with aflatoxin B_1 with minimum 1.3, maximum 24.5 and average ± SE 10.4± 5.1ppb, while the value of aflatoxin B_1 in Basterma samples minimum 1.2, 2.5 and average ± SE 2.3± o.4 ppb. Aflatoxin $B_1$was not detected in Frankfurter samples. There were 2 positive luncheon samples (24.5, 21.0 ppb) exceeding the maximal concentration (8 μg/kg), recommended by European Union regulation (European Union, 2001), for human consumption [56].

Therefore, it is of public health importance to evaluate the possible production of aflatoxin B_1 during meat processing and ageing. Few studies were carried out but they all demonstrated that the frequency of contamination of processed meat with aflatoxin B_1 is low and that the level of toxin within meat is usually below 10 ng /g. However, it is not clear weather aflatoxin B_1 was produced during meat processing or was present before at the residual level in muscles. Indeed, it seems that there is no relationship between the presence of toxigenic strains of *A. flavus* and aflatoxin contamination of meat samples. The frequent contamination of spices and additives used in such meat processing may also represent a source of mycotoxin. Moreover, it has been demonstrated that the use of spices contaminated with toxigenic mould strains as ingredient in meat products making may lead to a secondary contamination of the final product with aflatoxins [8, 55].

Among the increased demand of the meat products, it is of important to make these products of sanitary quality, they must be free from hazardous microorganisms or when present should be at a safe low level. The information given by the achieved results proved that most of the examined meat products are contaminated with mould, yeast, *S. aureus* and *Cl. perfringens* and aflatoxin B_1. Also the incidences of hazardous microorganisms like *S. aureus, Cl. perfringens* and presences aflatoxin B_1 are considered objectionable, as they render the product of inferior quality and unfit for consumption.

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