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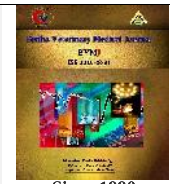

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Microbial Aspect of some processed meat products with special reference to aflatoxins

Khalifa E. Ahmed Abuzaid[1], Fahim Shaltout[2], Ramadan Salem[3], Eman M. El-Diasty[3]

[1] *HSEQ Supervisor in TSEBO, Egypt*

[2] *Department of Food Control, Faculty of Veterinary Medicine, Moshtohor, Benha University, Egypt, and Member of the Egyptian Organization for Standardization and Quality, and Meat Hygiene Committee*

[3] *Mycology Department, Animal Health Research Institute Dokki, Giza.*

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ABSTRACT

This work was performed to evaluate the microbial aspect of some processed meat products sold in local markets in Kaliobia governorate. Eighty random samples of meat products represented by sausages and Kofta (40 of each) were purchased from different shops at Kaliobia governorate to determine their microbiological criteria. All the examined samples were contaminated with different types of microorganisms with the mean values of $11\times10^6 \pm 5.4\times10^6$, and $2.04\times10^6 \pm 0.12\times10^6$ cfu/g for aerobic bacterial counts; $6.7\times10^3 \pm 0.3\times10^3$,and $1.2\times10^3 \pm 0.16\times10^3$ for coliforms;$1.1 x 10^3 \pm 0.14 x 10^3$ and $1.4 x 10^3 \pm 0.27 x 10^3$, total mould count; and $0.52 x 10^3 \pm .08 x 10^3$ and $0.47 x 10^3 \pm .07 x 10^3$ total yeast count, respectively. The incidence of isolated mould species in the examined sausage was higher than that in kofta samples. The average concentration of aflatoxins in sausage was 19.8 ± 2.5 with frequency and incidence of 13 (32.5%), while in kofta was 11.5 ± 3.3^a with frequency and incidence of 12(30%). *Aspergillus* and *Penicillium* species were the most prevalent species recovered from the examined meat product samples. The isolated *A. flavus* strains were screened for aflatoxin production. The public health significance of isolated microorganisms as well as recommended hygienic measures to keep meat products safe for human consumption were discussed as the result showed that the unhygienic and poor sanitary conditions of meat products.

1. INTRODUCTION

Meat and meat products are the most palatable and desirable foods for human being, as they are important source of animal protein, fat, essential amino acids, minerals, vitamins and other nutrients (Zafar *et al.*, 2016). On the other hand, they are considered as ideal culture medium for growth of many organisms because of the high moisture, high percentage of nitrogenous compounds, plentiful supply of minerals, some fermentable carbohydrates (glycogen) and favorable pH for most microorganisms resulting in their spoilage, economic losses, foodborne infections in human and health risk (Komba *et al*., 2012).

The bacterial contamination and hygienic measures during meat production can be measured using the aerobic plate count and coliforms (Hamed *et al*., 2015). Aerobic plat count is used as indicator for bacterial population, but it cannot differentiate types of bacteria. Aerobic plate counts can be useful to indicate quality and shelf life of certain food items. Sources of coliforms in meat are solid hands, knives used for cutting and contaminated water. Fecal coliforms had been used as indicator for fecal contamination of the meat (Shaltout *et al*., 2019).

Meat products may be contaminated with fungi which may occur during the transportation, storage and handling processes of meat (Nasser, 2015).

Contaminated meat products may constitute a public health hazard (Datta *et al*., 2012 and Hamed *et al*., 2015).

Fungi may cause three basic types of diseases which are mycosis, allergy and mycotoxicosis. A mycosis is defined as invasion of living tissue by fungi, while an allergy is a hypersensitivity to fungal antigen and mycotoxicosis is a toxic manifestation resulting from ingestion or exposure to fungal metabolites (El-Tawab., 2014). Mycotoxins, particularly aflatoxin (AF), fumonisin (FUM), and deoxynivaenol (DON), may impair child growth. Although these toxins have distinct actions, they all mediate intestinal damage through inhibition of protein synthesis (AF, DON), increase in systemic pro-inflammatory cytokines (DON) and inhibition of ceramide synthesis (Laura *et al*., 2012).

Chronic exposure to aflatoxin well above the FDA guideline 20 (ppb) affects many organs; however, the major targets is the liver. Aflatoxins are hepatotoxic in humans and animals, Food exposed to AFs and resulting in aflatoxicosis can ranged from acute to chronic, and illness can ranged from mild to severe, including development of cirrhosis (severe liver damage) and may result in development of liver cancer. Aflatoxin B_1 is the most potent known natural carcinogen (FDA, 2013).

As the level of meat contamination of meat and its products with different foodborne pathogens constitutes serious problems for consumers, so, the current study was conducted

* Corresponding author: Eman M. El-Diasty, Mycology Department, Animal Health Research Institute Dokki, Giza.

to microbial evaluation of some meat products with special attention to detection of aflatoxins.

2. MATERIAL AND METHODS

2.1. Sample collection

A total of 80 random samples from sausage and kofta (40 of each) were purchased from different shops at Kaliobia governorate. Each sample was kept in a separate sterile plastic bag and put in an icebox then transferred to the laboratory under complete aseptic conditions without undue delay and subjected to bacteriological and mycological examination.

2.2 Methods

2.2.1. Bacteriological examination

2.2.1.1. Determination of Aerobic Plate Count APC/g using standard plate count following (FDA, 2001)

2.2.1.2.Determination of coliform count by the surface plating method of using Violet Red Bile agar medium (ICMSF, 1996) .

2.2.2. Mycological examination

2.2.2.1. Determination of total mould and yeast count

The collected samples were prepared according to the technique recommended by ISO, 217-1-2:2008. The isolated fungi were identified according to macro and microscopic characteristics as described by Pitt and Hoching (2009), while yeast isolates were identified according to some complementary tests used for final identification of the isolates as recommended by Kurtzman *et al*. (2003) and Pitt and Hoching (2009).

2.2.2.2. Detection of aflatoxins residues in sausage and kofta samples by using fluorimeter method: (AflaTest Fluorometer Instruction Manual, 2014).

3. Statistical analysis

Data were analyzed using the descriptive statistic SPSS (Version 20). Differences in mean of analyzed data were considered significant at P < 0.05.

3. RESULTS

Results showed in Table (1) indicated the total aerobic bacterial count in the examined kofta samples, where the total aerobic bacterial count ranged from 10×10^3 to 5×10^7 with a mean value of $2.04\times10^6 \pm 0.12\times10^6$ cfu/g , while sausage samples ranged from 2.4×10^3 to 15×10^7 with a mean value of $11\times10^6 \pm 5.4\times10^6$ cfu/g.

Table 1 Statistical analytical results of Aerobic Plate Count (CFU/g) in the examined samples of meat products (n=40).

Meat products	Count (CFU/g)		
	Min.	Max.	Mean ± SE
Kofta	$1.0x10^4$	$5.0x10^7$	$2.04x10^6 \pm 0.12x10^{6a}$
Sausage	$2.4x10^3$	$15.0x10^7$	$11.00x10^6 \pm 5.4x10^{6a}$

(a): Means within the same column followed by different letters are highly significantly different (*P* < 0.05).

In Table 2, the results showed that coliform count ranged from 7.9×10^2 to 0.17×10^4 with a mean value of $1.2\times10^3 \pm0.16\times10^{3a}$ in kofta samples, while in sausage sample ranged from 8.9×10^2 to 3.5×10^4 with a mean value of $6.7\times10^3\pm 0.3\times10^3$ cfu/g.

Results achieved in table (3) indicated that the incidence of mould in the examined meat samples were (62.5%) and (82.5%) for the examined samples of Kofta, and sausage, respectively. Furthermore, the mean value of total mould count (cfu/g) in the examined sausage, and Kofta samples were $1.1x\ 10^3 \pm 0.14\ x\ 10^3$ and $1.4\ x\ 10^3 \pm 0.27\ x\ 10^3$, respectively.

Regarding the results recorded in the tables (3), it revealed that the incidence and total mould count of examined samples of sausage had a minimum count $1.0x10^2$ and maximum $4.2x10^3$ with a mean value of $1.1x10^3 \pm 0.14x10^3$ (cfu/g).

The results achieved in table (4) revealed that the incidence of yeast contamination in the examined kofta, and Sausage samples were 28 (70%), and 25 (62.5%). Furthermore, the mean value of total yeast (cfu/g) in the examined kofta and Sausage samples were: $.47\ x\ 10^3 \pm .07\ x\ 10^3$ and $.52\ x\ 10^3 \pm .08\ x\ 10^3$.

Table 2 Statistical analytical results of coliform count (CFU/g) in the examined samples of meat products (n=40).

Meat products	Incidence of contamination		Count (CFU/g)		
	No.	%	Min.	Max.	Mean ± SE
Kofta	6	15*	$7.9x10^2$	$0.17x10^4$	$1.2x10^3 \pm 0.16x10^{3a}$
Sausage	10	25*	$8.9x10^2$	$3.5x10^4$	$6.7x10^3 \pm 0.3x10^{3a}$
Total	16	20**			

*. Incidence of positive coliform samples in relation to total number of each product (40). **. Incidence of positive coliform samples in relation to total number of samples (80). (a): Means within the same column followed by different letters are highly significantly different (*P* < 0.05).

Table 3 Statistical analytical results of mould counts (CFU/g) in the examined samples of meat products (n=40).

Meat products	Incidence of contamination		Mould Count (CFU/g)		
	No.	%	Min.	Max.	Mean ± SE
Kofta	25	62.5*	$0.7x10^2$	$5.2x10^3$	$1.4x10^3 \pm 0.27x10^{3a}$
Sausage	33	82.5*	$1.0x10^2$	$4.2x10^3$	$1.1x10^3 \pm 0.14x10^{3a}$
Total	58	72.5**			

*. Incidence of positive mould samples in relation to total number of each product (40). **. Incidence of positive mould samples in relation to total number of samples (80). (a): Means within the same column followed by different letters are highly significantly different (*P* < 0.05).

Table 4 Statistical analytical results of yeast counts (CFU/g) in the examined samples of meat products (n=40).

Meat products	Incidence of contamination		Yeast Count (CFU/g)		
	No.	%	Min.	Max.	Mean ± SE
Kofta	28	70.0	$1.0x10^2$	$1.4x10^3$	$0.47x10^3 \pm 0.07x10^{3a}$
Sausage	25	62.5*	$0.9x10^2$	$1.7x10^3$	$0.52x10^3 \pm 0.08x10^{3a}$
Total	53	66.2**			

*. Incidence of positive yeast samples in relation to total number of each product (40). **. Incidence of positive yeast samples in relation to total number of samples (80). (a): Means within the same column followed by different letters are highly significantly different (*P* < 0.05).

Results achieved in table (5) declared that 7 mould genera could be isolated and identified from the examined samples. The identified mould genera were *Acremonium, Aspergillus, Cladosporium, Chaetomonium, Geotrichum, Penicillium, and Talaromyces* species. The incidence of identified mould isolated from the examined sausage and kofta was (40 and 25%) for A.flavus, (32 and 18%) for *A.niger*, (15 and 5%) for *A. ustus*, (5 and 2.5%) for *A.terreus*, (2.5 and 0 %) for *A.*

flavipes, (and 0 and 7.5%) for *A. clavatus*, (10 and 0%) for *A. fumigatus*, (0 and 2.5%) for *A. nidulens*, (7.5 and 5%) for Acremonium spp, (2.5 and 7.5 %) for C*l. Cladosopridiae,* (0 and 5%) for *Chaetomonium globosum*, (12.5 and 7.5%) for *P. citreonigrum*, (5 and 2.5%) for *P. oxalicum*, (5 and 7.5%) for *P. fellutanum*, and (0 and 12.5%) for *P. chrysogenum*, (0 and 7.5%) for *T. trachycpermus*, and (5 and 15%) for *Geotrichum candidum* .

The incidence of identified yeast isolated from the examined sausage and kofta samples were (5 and 2.5%) for *Candida pseudotropicalis*, (17.5 and 10%) for *C. homlii*, (12.5 and 17.5%) for *C. famata*, (5 and 10%) for *C. gullermondii*, (25 and 22.5%) for Rhodotorula spp., (12.5 and 2.5%) for Saccharomyces spp., (5 and 7.5%) for Torulopsis spp., *C. valida* was only present in the examined sausage sample with incidence of 10% (Table 6).

Table 5 Incidence of the identified fungal strains isolated from the examined samples of meat products (n=40).

Mould spp.		Sausage		Kofta	
		No.	%	No.	%
A.	Aspergillus spp.				
	- *A. flavus*	16	40.0	10	25.0
	- *A. flavipes*	1	2.5	0	0
	- *A. niger*	32	80.0	18	45.0
	- *A. ustus*	6	15.0	2	5.0
	- *A. terreus*	2	5.0	1	2.5
	- *A. clavatus*	0	0.0	3	7.5
	- *A. fumigatus*	4	10.0	0	0.0
	- *A. nidulens*	0	0	1	2.5
B.	Acremonium spp.	3	7.5	2	5.0
C.	Cladosporium spp.				
	- *Cl. Cladosporidiae*	1	2.5	3	7.5
D.	Chaetomonium spp.				
	- *Chaetomonium globosum*	0	0	2	5.0
E.	Penicillium spp.				
	- *P. citreonigrum*	5	12.5	3	7.5
	- *P. oxalicum*	2	5.0	1	2.5
	- *P. fellutanum*	2	5.0	3	7.5
	- *P. chrysogenum*	0	0	5	12.5
F.	Talaromyces spp.				
	- *T. trachycpermus*	0	0	3	7.5
G.	Geotrichum spp.				
	- *G. candidum*	2	5.0	6	15.0

Table 6 Incidence of the identified yeast strains isolated from the examined samples of meat products (n=40).

Yeast spp.		Sausage		Kofta	
		No.	%	No.	%
A.	Candida spp.				
	- *C. pseudotropicalis*	2	5	1	2.5
	- *C. holmii*	7	17.5	4	10.0
	- *C. famata*	5	12.5	7	17.5
	- *C. valida*	4	10.0	0	0.0
	- *C. gullimondii*	2	5.0	4	10.0
B.	Rhodotrulla spp.	10	25.0	9	22.5
C.	Saccharomyces spp.	5	12.5	1	2.5
D.	Torulopsis spp.	2	5.0	3	7.5

Table (7) revealed that the incidence and average concentration of aflatoxins (µg/kg) in the examined samples of meat products. The average conc. of aflatoxins in kofta was 11.5 ±3.3^a with frequency and incidence of 12 (30%), while in sausage with an average concentration of 19.8±2.5^a with frequency and incidence of 13(32.5%).

4. DISUCSSION

Meat and meat products are considered as major vehicles of most reported food poisoning outbreaks. Therefore, it is important to use the microbiological criteria to determine the quality of such products.

Table 7 Incidence and mean concentration level of aflatoxins in the examined samples of meat products (n=40).

Meat products	Incidence		Mean ± SE
	No.	%	
Kofta	12	30.0*	11.5 ± 3.3^a
Sausage	13	32.5*	19.8 ± 2.5^a
Total	25	31.25**	

*. Incidence of positive aflatoxin samples in relation to total number of each product (40). **. Incidence of positive aflatoxin samples in relation to total number of samples (80). (a): Means within the same column followed by different letters are highly significantly different ($P < 0.05$).

Processed meats may be contaminated with several types of microorganisms from different sources during the period of slaughtering, preparation, processing and cooking (Narasimha and Ramesh, 1988).

Concerning kofta samples, nearly similar results were obtained by *El-Taher- Amna (2009)* who recorded that the mean value of APC was 1.26×10^6 for kofta. Sausage samples ranged from 2.4×10^3 to 15×10^7 with a mean value of 11×10^6 ±5.4×10^6.Nearly similar results were obtained by Elmossallami (2003) who recorded that aerobic plate count of sausage samples was 9.3 ×10^6 organisms/g. Also, these results counts were higher than recorded by El-Maghraby-Marwa (2014); Ahmed- Alyaa (2015) and Hamed *et al.* (2015). Lower results were recorded by Hamouda (2005) who said that the mean value of aerobic plate count of sausage samples was 5×10^5.

The total Aerobic bacterial count of any food articles is not only a sure indicator of its safety for consumption, yet it is of importance in judging the hygienic conditions under which it has been processed and handled (Saad, 1976).

The results of coliforms count are higher than those reported by El-Maghraby-Marwa (2014); Ahmed- Alyaa (2015) and Hamed *et al.* (2015).

The presence of *Coliforms* in meat and meat products indicates a potable faecal source of contamination which begins from slaughterhouse as a result of skinning of animals by knives and workers, also during evisceration. Contamination may come from animal intestine, air and water used for washing and rinsing of carcasses (Gaafer, 2009).

Incidence of mould in the examined meat samples were (62.5%) and (82.5%) for the examined samples of Kofta, and sausage respectively. The obtained results were nearly similar to those recorded by El-Diasty and Wahba (2008) who recorded that the incidence of mould in sausage samples was (80%), Sausage had a higher incidence as they are raw not heat treated beside the addition of some meat additives. The obtained results were nearly similar to that obtained by Lamada and Nassif (2008) who reported an incidence of 60% in kofta. However, the incidence was lower than Hussein (2008) who reported that the incidence of mould in kofta (93.3%).

These results were nearly higher than Shaltout and Salem (2000); Abu Zaid (2015) and Morshdy *et al.* (2015) who mentioned that mean value of total mould count of sausage samples was $11\times10^2 \pm 3\times10^2$. While higher figure were reported by Shaltout (1996); Maha and Sohad (2005); El-Diasty and Wahba (2008) and Naas *et al*. (2009), who mentioned that examined fresh beef sausage samples had mould count $2.3\times10^6 \pm 2.7\times10^5$ cfu/g .

The obtained results of the examined sausage samples were nearly similar to those recorded by Shaltout and Salem (2000); and Hussein (2008).Meanwhile, the results of our present study were higher than those reported by EL- Tabiy (2006) who found that the mean value of total mould counts in examined sausage samples were 1.0 X $10^2 \pm 0.33$ X 10 (cfu/g). The obtained results were lower than those reported by Eleiwa and El-Diasty (2014). According to the permissible limits stipulated by EOS (2005) for total mould and total yeast total yeast count (Free) in case of kofta, and sausage. (Table 8, Fig. 1).

Table 8 Acceptability of the examined meat products based on EOS legislations.

Product	EOS standard No.	EOS legislations		Accepted samples		Rejected samples	
		Parameter	PL. (CFU/g)	No.	%	No.	%
Kofta	1973\2005	APC	$>10^6$	29	72.5	11	27.5
		Coliform	$>10^2$	34	85.0	6	15.0
		Mould & Yeast	Free	12	30.0	28	70.0
Sausage	1972\2005	APC	$>10^6$	28	70.0	12	30.0
		Coliform	$>10^2$	30	75.0	10	25.0
		Mould & Yeast	Free	7	17.5	33	82.5

PL.: Permissible Limit.

The results of mould identification agreed with those obtained by Seham- Ismail *et al*. (2013) who reported that 7 mould genera were identified in the examined samples. The identified mould genera were *Aspergillus, Penicillium, Eupencillium, Cladosporium and Byssochlamys nivea*. The predominant species were Aspergillus, and Penicillium. The frequencies of isolated mould genera in examined samples were *A. niger* 10 (26.3 %), and A. *flavus* 7(18.4%).

Yeasts contribute a small but permanent part of the natural microbiota on meat. The results of identified yeast isolated from the examined sausage and kofta samples come in accordance with those recorded by Shaltout (1996), Samaha (2013), and Eleiwa and El-Diasty (2014) whom reported that mentioned that 7 species belonging to 4 yeast genera were isolated from examined sausage samples were *Candida albicans*, *Candida kruesi, Candida neaformans*, *Candida tropicalis*, *Cryptococcus spp., Rhodotorula spp.* and *Saccharomyces cerevisiae*. While *C. famata*, *C. pelliculosa*, *C. tropicals, C. parapasilosis, Cryptococcus spp.*, *Rhodotorula spp*. and *Torulopsis spp*. were isolated from minced meat (Salem *et al.,* 2015).

These findings may be attributed to use of unsterilized spices (untreated food additives) which usually carry mould spores used in manufacture of this meat products specially both of oriental sausage and kofta as this fresh products usually manufacture under unhygienic conditions in addition to use of inferior quality raw materials.

Regarding the incidence and average concentration of aflatoxins (µg/kg), higher values were detected by Refai et al. (2003) and EL-diasty (2013). These different mean values of aflatoxins residues may be related to the amount of additives contamination with aflatoxin and the amount of aflatoxin residues which may be present in animal muscles. At the same time, the mean values of detected aflatoxins in the examined samples were lower than the maximum permissible limit recommended by commission regulation (EC), the maximum levels of aflatoxins (aflatoxins B_1, B_2, G_1, G_2, and M_1) are 10-15µg/ kg and B_1 is 5 µg/kg in as commission Regulation (EC) No. 1881/2006..

5. CONCULOSIONS

The result demonstrates that the unhygienic and poor sanitary conditions under which the meat and meat products where handled and processed are not acceptable from the sanitary point of view. Also, the undesirable level of contamination which might have acquired from the environment and agents and considered as a major cause in the spoilage of meat products, leading to great economic losses and constitute a public health hazards by production of wide variety of mycotoxins and to obtain wholesome, safe and sound meat products, the principles of Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Point (HACCP) must be adopted.

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