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

Seham, A. Ismail, Amal, A. Shehata and E.M. El-diasty

Giza Laboratory
Food Hygiene Department
Mycology Department - Animal Health Research Institute, Dokki, Egypt

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, Penicillium, 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%), Rhodotorula mucilaginosa 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 mucilaginosa 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 B$_1$ minimum 1.3, maximum 24.5 and average ± SE 10.4 ± 5.1 ppb, while the value of aflatoxin B$_1$ in Basterma samples minimum 1.2, 2.5 and average ± SE 2.3 ± 0.4 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 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

Corresponding Author: E.M. El-diasty, Mycology Department - Animal Health Research Institute, Dokki, Egypt.
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 aflatoxinB1, content residues and effect of some food processing methods on the stability of mycotoxin aflatoxinB1 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 enterotoxigenic 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 enterotoxigenic 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_ 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)

<table>
<thead>
<tr>
<th>Meat products</th>
<th>Mould</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Frankfurter</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Luncheon</td>
<td>23</td>
<td>92</td>
</tr>
<tr>
<td>Basterma</td>
<td>22</td>
<td>88</td>
</tr>
</tbody>
</table>

N= number of samples

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

<table>
<thead>
<tr>
<th>Mould genera</th>
<th>Frankfurter</th>
<th>Luncheon</th>
<th>Basterma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Aspergillus species A. niger</td>
<td>2</td>
<td>15.4</td>
<td>10</td>
</tr>
<tr>
<td>A. flavus</td>
<td>4</td>
<td>30.8</td>
<td>7</td>
</tr>
<tr>
<td>A. ochraceus</td>
<td>1</td>
<td>7.7</td>
<td>-</td>
</tr>
<tr>
<td>A. gluacus</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pencicillium species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. simplicissimum</td>
<td>2</td>
<td>15.4</td>
<td>2</td>
</tr>
<tr>
<td>P. chrysogenum</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. fumiculosum</td>
<td>1</td>
<td>7.7</td>
<td>-</td>
</tr>
<tr>
<td>P. nalgiovese</td>
<td>2</td>
<td>15.4</td>
<td>-</td>
</tr>
<tr>
<td>P. digitatum</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. raistrickii</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. rugulosum</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P.olsonii</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eupencillium species</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Eurotium species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. chevalier</td>
<td>1</td>
<td>7.7</td>
<td>-</td>
</tr>
<tr>
<td>Mucor</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Cladosporium species</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Byssoschlamys nivea</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

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 Byssoschlamys 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. nalgiovese 2 (15.4%) and A. ochraceus, P. fumiculosum and E. chevalier1 (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 Byssoschlamys 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%), 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 Pencillium 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...
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 mucilaginosa 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%), R. mucilaginosa 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<sub>1</sub> during meat processing and ageing.

The results recorded in table (6) revealed that 5 (20%) samples of luncheon are contained with aflatoxin B<sub>1</sub>, with minimum 1.3, maximum 24.5 and average \pm 10.4 \pm 5.1 ppb, while the value of aflatoxin B<sub>1</sub> in Basterma samples minimum 1.2, 2.5 and average \pm 2.3 \pm 0.4 ppb. Aflatoxin B<sub>1</sub> was not detected in Frankfurter samples. There were 2 positive luncheon samples (24.5, 21.0 ppb) exceeding the maximal concentration (8 \mu g/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<sub>1</sub> 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<sub>1</sub> is low and that the level of toxin within meat is usually below 10 ng /g. However, it is not clear weather aflatoxin B<sub>1</sub> 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 B₁. Also the incidences of hazardous microorganisms like *S. aureus*, *Cl. perfringens* and presences aflatoxin B₁ are considered objectionable, as they render the product of inferior quality and unfit for consumption.

REFERENCES


42. Abdel-Rahman, N.M., 1995. Pathogenic yeasts in meat products. M.D. Thesis, Faculty of Veterinary Medicine, Cairo University.


47. El-Khateib, T., 1997. Microbiological status of Egyptian salted meat (Basterma) and fresh sausage. Journal of Food Safety, 17: 141-150.